PB-216 721

PROCEEDINGS: NORTHWEST SHELLFISH SANITATION RESEARCH PLANNING CONFERENCE, 1966

W. J. Beck, et al

National Center for Urban and Industrial Health
Cincinnati, Ohio

1967

DISTRIBUTED BY:

NTIS

National Technical Information Service
U. S. DEPARTMENT OF COMMERCE
5285 Port Royal Road, Springfield Va 22151

ENVIRONMENTAL HEALTH SERIES

This SERIES is published by the Bureau of Disease Prevention and Environmental Control of the Public Health Service to provide a centralized source of information on the scientific and technical studies performed by various programs within the Bureau dealing with the maintenance of a healthful environment for man. Individual reports in the series deal with the health factors involved in: air pollution; radiological contamination; and the urban and industrial environment including water resources, solid waste disposal, accidental injury, environmental engineering and sanitation, and occupational health hazards.

The general subject area of each report is indicated by several letters that appear in the publication number as follows:

AP----- Air Pollution
RH----- Radiological Health
UIH---- Urban and Industrial Health

Reports in the SERIES will be distributed on request as supplies permit. Requests should be directed to the National Center identified on the title page of each report.

PB 216 721

1966
PROCEEDINGS

Northwest Shellfish Sanitation Research Planning Conference

W.J. Beck and J.C. Hoff

Northwest Shellfish Sanitation Laboratory
Gig Harbor, Washington

Reproduced by
NATIONAL TECHNICAL
INFORMATION SERVICE
U S Department of Commerce
Springfield VA 22151

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service
Bureau of Disease Prevention and Environmental Control
National Center for Urban and Industrial Health
Shellfish Sanitation Branch
Water Supply and Sea Resources Program
1967

Disclaimer Clause

The use of trade names of products in this publication are for example only and do not imply endorsement by the Public Health Service or the U. S. Department of Health, Education and Welfare.

Public Health Service Publication No. 999-UIH-1

PREFACE

Beginning in 1959, annual Northwest Shellfish Sanitation Research Planning Conferences have been held. This is the third annual conference to be formally published in this format. These Proceedings represent the tangible results of our efforts during the past year.

As in previous years, significant changes in physical facilities, research efforts and future plans have taken place. We hope that each of you finds the published material helpful in the performance of your responsibilities in the area of shellfish sanitation. Once again, we would like to thank you for your continued interest and participation in these conferences.

C. B. Kelly

CONTENTS

Page

PART I. RESEARCH PROGRESS REPORTS

Summarization of Activities, W. J. Beck 2

Research Projects in Progress, J. C. Hoff 3

Comparative Study of Several Indicator Organisms in Samples Collected from Three Northwest Watersheds, G. J. Vasconcelos, R. D. Lee and R. F. Coene 5

Studies on the Incidence of Clostridium botulinum Type E in Some West Coast Estuarine Areas, T. H. Ericksen 19

Accumulation and Retention of Clostridium botulinum Type E Spores by Manila Clams, T. H. Ericksen 31

Studies on Wet Storage of Oyster and Clam Shellstock, W. Jakubowski and G. J. Vasconcelos 41

Accumulation of Bacteria by Two Species of West Coast Shellfish Maintained in an Estuarine Environment, W. Jakubowski . 54

Chemical and Biological Analysis of Shellfish and Seawater at Site of Northwest Shellfish Sanitation Research Center, W. J. Beck 61

PART II. PROPOSED RESEARCH ACTIVITIES FOR FISCAL YEAR 1967

Ecological Studies 77

Accumulation and Storage of West Coast Shellfish Polluted with Clostridium botulinum Type E Spores 78

Studies on Wet Storage of Oyster and Clam Shellstock 78

Depuration of West Coast Hard-shell Clams 79

Accumulation and Elimination of Poliovirus by the Olympia Oyster (Ostrea lurida) 80

Cooperative Studies at the NWSSRC Site 81

Evaluation of a Plating Technic for Enumeration of Fecal Coliform Organisms 82

Research on Gonyaulax Toxins 82

Page

PART III. APPENDIX

Agenda 85

Attendance Roster 87

PART I

RESEARCH PROGRESS REPORTS

SUMMARIZATION OF ACTIVITIES

W. J. Beck

The transitional period of the Northwest Shellfish Sanitation Laboratory has continued during the past year. The number of personnel attached administratively to the laboratory has more than doubled. A cooperative study with the PHS Division of Water Supply has reemphasized the need for research on indicator bacteria plus methods for the enumeration of viruses from large quantities of water. Certain research projects such as anaerobic bacteriology, enumeration and identification of viruses in shellfish and wet storage studies have been continued.

Several publications by this station have been completed or accepted for publication. The 1964 and 1965 Proceedings of this Conference are now available. A note on the Application of an Endpoint Dilution Method for Bacteriophage Assay was published in the May 1966 issue of Applied Microbiology. Two papers on storage of oysters and clams have been accepted for publication in the Journal of Food Science. Papers on our work on accumulation and elimination of bacteria and bacteriophage were presented at the recent National Depuration Conference. These will be published in the Proceedings of this Conference.

With the announcement of the site selection for the Northwest Shellfish Sanitation Research Center, administrative and technical activities have rapidly increased. Many conferences have been held with agencies and institutions interested in developing a Regional Marine Science Complex at the Manchester site. Although background data have been collected since February 1965, development of the site has not really begun. Arrangements are now being made for the various phases of construction necessary for the center.

During the past year we have been very fortunate in the cooperation shown by the various federal and state agencies, universities and industry. Only through this coordinated effort have we been able to augment a small field station into a research center. We wish to thank each of you for your assistance in the past and look forward to your guidance in the future.

RESEARCH PROJECTS IN PROGRESS

J. C. Hoff

In addition to the formal reports to be presented at this conference, several other research projects are in progress at the laboratory. Since these projects have not reached a point at which a report can be presented, the results will be summarized.

Work on a pilot scale depuration tank design is underway. The necessary small tanks, flow rate control apparatus, and equipment necessary for determining dissolved oxygen, salinity, temperature and turbidity have been constructed or obtained. Only one experiment has been conducted and no statement regarding flow rates necessary can be made at this time.

There have been several changes in the virology research program in the past year. At the 1965 Research Planning Conference, a study of the effects of ultraviolet radiation of viruses was proposed. A review of the literature was made and a report incorporating this background material was prepared. A meeting of the virologists from the three shellfish sanitation research laboratories was held March 24 and 25 at Dauphin Island, Alabama. At this meeting it was determined that this phase of the research should be transferred to the Gulf Coast Shellfish Sanitation Research Center since personnel there were doing other work which related to the problem of U. V. inactivation.

Since then efforts here have been directed toward the study of the accumulation and elimination of poliovirus by Olympia oysters. The experimental system, including aquaria and dosing devices, has been designed and constructed. Stocks of poliovirus have also been prepared but no experiments have been conducted.

Efforts are also being made to develop improved methods for the concentration of viruses from water. Using the gauze pad method, a flow through type sampler has been developed. These samples are currently being used in connection with the Northwest Watershed Project. Laboratory evaluations on the efficiency of the device are also being made. One experiment has been conducted using known levels of poliovirus. The samples have not been examined.

In former years, much of the work presented at these conferences has been the result of large volume routine experiments employing well established standardized methods. Several of these investigations are only now reaching completion as publications in professional journals. We are still engaged in some projects of this type such as the wet

storage studies. However, our research efforts in other areas such as Clostridium botulinum and virology have necessitated changes in equipment, development of new methods, and development of specialized proficiencies by the research staff. Accomplishments of this type do not always lend themselves to presentation as formal reports. Therefore, while we have made substantial progress in these areas, the results are not as visible or discernible as in some of the other studies.

COMPARATIVE STUDY OF SEVERAL INDICATOR ORGANISMS IN SAMPLES COLLECTED FROM THREE NORTHWEST WATERSHEDS

G. J. Vasconcelos, R. D. Lee,[1] and R. F. Coene[1]

INTRODUCTION

The twelfth edition of APHA Standard Methods for the Examination of Water and Wastewater (1965) describes three bacteriological procedures commonly used for estimating the number of coliform, fecal coliform and fecal streptococcus organisms in fresh water. These three indices are often used in estimating the degree of bacterial contamination in rivers and streams. Of the three, the coliform group is still recognized as the standard indicator of fecal contamination. However, in the 12th edition of Standard Methods (1965) the MPN test for fecal coliforms has been included as a standard procedure for differentiating those coliform bacteria of fecal origin from the broader coliform group.

The frequent attention given to the potential value of the fecal streptococcus MPN test as an indicator of pollution has prompted its inclusion in the 12th edition of Standard Methods (1965). However, it remains a tentative method until further research is completed. As a supporting aid to the MPN methods, the inclusion of a direct plating method such as the 20 C plate count has also been recommended.

Various studies have been conducted in the past few years in comparing the relative relationship and distribution of coliform, fecal coliform and fecal streptococcus organisms in rivers and streams. Kaushik and Bewtra (1965) reported a coliform/enterococcus geometric mean ratio of 25:1 and a positive correlation of +0.787 in samples obtained from a river source. However, Hanes, Delaney and O'Leary (1965) concluded that no definite equation could be developed between enterococci and the coliform group or Escherichia coli because the relationship varies with the source of contamination.

Bacteriological data collected from three Northwest watersheds over a six month period were subjected to a graphical, statistical analysis in a manner described by Velz (1951). Results from this analysis were then compared as a means of evaluating the relative reliability and selectivity of the coliform, fecal coliform and fecal streptococcus MPN methods.

---

[1]Project Director and Assistant Project Director respectively, Northwest Watershed Project, Public Health Service.

COMPARATIVE STUDY OF SEVERAL INDICATOR ORGANISMS IN SAMPLES COLLECTED FROM THREE NORTHWEST WATERSHEDS

G. J. Vasconcelos, R. D. Lee,[1] and R. F. Coene[1]

INTRODUCTION

The twelfth edition of APHA Standard Methods for the Examination of Water and Wastewater (1965) describes three bacteriological procedures commonly used for estimating the number of coliform, fecal coliform and fecal streptococcus organisms in fresh water. These three indices are often used in estimating the degree of bacterial contamination in rivers and streams. Of the three, the coliform group is still recognized as the standard indicator of fecal contamination. However, in the 12th edition of Standard Methods (1965) the MPN test for fecal coliforms has been included as a standard procedure for differentiating those coliform bacteria of fecal origin from the broader coliform group.

The frequent attention given to the potential value of the fecal streptococcus MPN test as an indicator of pollution has prompted its inclusion in the 12th edition of Standard Methods (1965). However, it remains a tentative method until further research is completed. As a supporting aid to the MPN methods, the inclusion of a direct plating method such as the 20 C plate count has also been recommended.

Various studies have been conducted in the past few years in comparing the relative relationship and distribution of coliform, fecal coliform and fecal streptococcus organisms in rivers and streams. Kaushik and Bewtra (1965) reported a coliform/enterococcus geometric mean ratio of 25:1 and a positive correlation of +0.787 in samples obtained from a river source. However, Hanes, Delaney and O'Leary (1965) concluded that no definite equation could be developed between enterococci and the coliform group or Escherichia coli because the relationship varies with the source of contamination.

Bacteriological data collected from three Northwest watersheds over a six month period were subjected to a graphical, statistical analysis in a manner described by Velz (1951). Results from this analysis were then compared as a means of evaluating the relative reliability and selectivity of the coliform, fecal coliform and fecal streptococcus MPN methods.

---

[1]Project Director and Assistant Project Director respectively, Northwest Watershed Project, Public Health Service.

METHODS AND MATERIALS

Samples:

Raw fresh water samples were collected from 41 stations located on three watersheds in the Northwest area. All stations were visited at least once each week. Sampling started in December of 1965 and continued through June of 1966. All water samples for bacteriological examination were collected and transported to the laboratory in a manner described by Standard Methods (1965).

Bacteriological Examination:

Once the samples were received at the laboratory they were immediately removed from their insulated containers and shaken vigorously 25 times. Graduated quantities of 100, 10, 1 and 0.1 ml were then inoculated into a series of azide dextrose and lauryl sulfate tryptose broth tubes. All samples were examined bacteriologically by the five tube MPN method for coliforms, fecal coliforms and fecal streptococci in accordance with Standard Methods (1965). In addition, these same samples were examined by the 20 C plate count.

Additional procedures were carried out on certain samples selected on a weekly basis. This involved the isolation of cultures from positive EC tubes by streaking on EMB agar and subsequent determination of coliform type by the "IMViC Test." From these results a completed fecal coliform MPN was computed.

Fecal streptococcus MPN's were determined using the multitube technic described in Standard Methods (1965). The presence of turbidity in inoculated tubes of azide dextrose broth, recommended by Mallmann and Seligmann (1950), was considered a positive presumptive test. Growth from positive azide dextrose broth tubes was transferred to ethyl violet azide broth (EVA) described by Litsky, Mallmann, and Fifield (1955). The formation of a purple button or dense turbidity in EVA was considered a positive confirmatory test for fecal streptococci. From these results a confirmed fecal streptococcus MPN was determined.

Positive EVA tubes from samples selected on a weekly basis were carried through the completed test by streaking directly to M-enterococcus agar. If growth occurred on this medium typical red or pink colonies were picked to brain heart infusion broth (BHI). Each isolate was tested for ability to grow in BHI broth at 45 C and 10 C. Growth (turbidity) at both temperatures constituted a positive, completed test. From these results a completed fecal streptococcus MPN was computed.

During the latter course of study two innovations in the fecal streptococcus procedure were made: (1) One brand of EVA was replaced by another, which contained a higher concentration of ethyl violet and substituted biosate, a hydrolysate of plant and animal protein, for tryptose. (2) Because of a change in Standard Methods (1965), growth in BHI at 10 C was discontinued as a criterion for the completed test. Instead, growth in BHI plus 40% bile at 45 C and a negative catalase test were used as additional criteria.

Plate counts were determined by placing replicate 1 ml and 0.1 ml portions of each sample into sterile disposable petri dishes. Ten ml amounts of melted and cooled Standard Plate Count Agar were then added to each plate. All plates were incubated at 20 C and read at the end of 48 hours with the aid of a Quebec colony counter.

Data Analysis:

Data from the three watersheds were combined and analyzed as a group. Emphasis was placed on comparing the relative selectivity, reliability and variability of each group of indicator organisms in relationship to one another.

Confirmed and completed MPN values of each index were plotted on logarithmic probability paper in a manner described by Velz (1951). Limitations inherent in the MPN method from the practical standpoint precluded the enumeration of organisms in concentrations lower than 0.18/100 ml. A graphic illustration showing the degree of relationship between the confirmed and completed fecal coliform and fecal streptococcus MPN's was also made. Because of the low levels of organisms present a large number of MPN results were indeterminate (<0.18). The determinate results were included in the initial grouping of the data and were assigned plotting positions on the probability plots.

RESULTS

Calculations were based on 770 samples collected over a six-month period starting in early winter of 1965 and continuing through the early summer of 1966. During this period water temperatures recorded at various stations on the three watersheds ranged from 0.5 C to 15 C.

A comparison of MPN's of the three confirmed bacterial indices is plotted on probability paper in Fig. 1 and the results summarized in Table 1. Coliform MPN's at the 10, 50 and 90 percentile levels were 0.42, 2.9 and 20 respectively. Fecal coliform MPN's at these same three percentile levels were <0.18, 0.39 and 3.2 respectively. At the

50 and 90 percentile levels, the fecal streptococcus MPN's were somewhat higher than those of the fecal coliform group but lower than those of the coliform group. Fecal coliform and fecal streptococcus MPN's at the 10 percentile level were both indeterminately low.

A comparison on probability paper of completed fecal coliform and fecal streptococcus MPN's is shown in Fig. 2 and the results summarized in Table 2. The completed fecal coliform and fecal streptococcus MPN's at the 50 percentile level were 0.36 and 0.19 respectively. At the 90 percentile level these same MPN's were 2.8 and 1.3 respectively. The completed fecal coliform median shown on Table 2 was 0.45 whereas the completed fecal streptococcus MPN median was 0.20. The range and standard deviation of both tests was similar.

As an additional parameter in this study, 736 samples were examined by 20 C Standard Plate Count. Counts ranged from a low of 50 organisms/ml to a high of 1,800/ml with a mean value of 170. Since the plate counts were estimated on a per ml basis, and enumerated a wider spectrum of organisms than the various MPN methods employed, a detailed comparison of the two was not attempted.

Results of those samples where both confirmed and completed MPN's were determined for fecal coliforms and fecal streptococci are graphically compared in Figs. 3 and 4. A tabulation of this graphical data is shown in Table 2.

The confirmed and completed fecal coliform results showed comparable MPN's of 0.46 and 0.36 respectively at the 50 percentile level and 3.7 and 2.8 at the 90 percentile level. Moreover, the slopes of both distribution lines were in close proximity to one another, indicating a high degree of correlation between confirmed and completed tests. The median and range of both confirmed and completed tests shown on Table 2 were identical.

The completed fecal streptococcus MPN's were lower than the confirmed MPN's at both the 50 and 90 percentiles, denoting little correlation between confirmed and completed tests. The degree of correlation in this instance was not as high as that observed between the confirmed and completed fecal coliforms MPN's.

During the completed fecal streptococcus MPN test it was observed that a large number of positive EVA tubes transferred to M-enterococcus agar failed to produce typical pink or red colonies. The majority of these pinpoint colonies were produced from tubes of EVA where only turbidity had been recovered. Where purple buttons were found in the EVA tubes, typical colonies were generally grown on the M-enterococcus agar and subsquently positive at 45 C incubation and 40% bile.

In an attempt to visually illustrate the degree of discrepancy between confirmed and completed fecal coliform and fecal streptococcus MPN's, a bar graph based on 203 paired determinations from each index is shown in Fig. 5. One hundred and sixty (79%) of the 203 paired confirmed and completed fecal coliform MPN were in complete agreement. Of the remaining 43, some disagreed by as much as 80-90%. The majority, however, disagreed by 40 to 50%. Only 43 (21%) of the 203 paired confirmed and completed fecal streptococcus MPN values completely. Of the remaining 160, more than half disagreed by a factor of 80% or more.

DISCUSSION

The arrangement of bacteriological data and computations necessary for determining the probability equal to or less than for each group of index organisms has resulted in plots forming a straight line. This would indicate that the data have a normal distribution and in accordance with the theory of probability results are expected to be distributed in this manner (Velz, 1951).

Results from the confirmed test indicated that the coliform MPN's were on the average six to seven times higher than the fecal coliform MPN's and three to five times higher than the fecal streptococcus MPN's. These results also indicated that the fecal streptococcus MPN's were in most instances the same or higher than the fecal coliform MPN's. However, a reverse relationship was shown between fecal coliform and fecal streptococcus in a number of samples subjected to the more precise and selective completed test. Results from these completed tests showed that in most instances the fecal streptococcus MPN's were the same or lower than the fecal coliform MPN's.

Results from this study also showed a close correlation between confirmed and completed fecal coliform MPN's. In most instances the two separately computed MPN values were in complete agreement. In contrast to this, confirmed and completed fecal streptococcus MPN's did not agree completely and in most instances disagreed by a substantial margin.

This lack of correlation may have resulted from the inability of the confirmatory EVA medium to selectively inhibit streptococcus organisms of nonfecal origin. Organisms not inhibited by this medium and transferred to M-enterococcus agar would then fail to propagate.

The major disadvantage of the fecal streptococcus MPN technic in this study appears to be its lack of selectivity. The inability of the confirmatory medium to selectively inhibit nonfecal streptococci

organisms is evidenced by the discrepancy in counts observed between confirmed and completed MPN's. If the fecal streptococcus MPN test is to be included among the accepted standard tests for the examination of water, efforts should be made to correct this apparent discrepancy.

To date, the most reliable and selective indicator of fecal contamination in this study appeared to be the fecal coliform group enumerated by the elevated temperature EC test. If compared with the broader coliform group the standard indicator of fecal contamination at use in most laboratories, the two often parallel each other, but at different MPN levels. The discrepancy observed between confirmed and completed fecal streptococcus MPN's would tend to discourage its use as a singular indicator of fecal contamination when only confirmed MPN values are reported. However, if circumstances permit, its use in addition to coliform and fecal coliform tests for comparison may be advantageous.

COMMENTS BY PARTICIPANTS

Mr. Neufeld commented on the better agreement shown between confirmed and completed fecal coliform tests as compared with the confirmed and completed fecal streptococcus tests. He also inquired about the length of time required for completing fecal streptococcus tests and the use of lagarithmic plots of MPN's. Mr. Vasconcelos pointed out that MPN values are logarithmically normal. Mr. Michener inquired about the use of BHI broth in the fecal streptococcus test. Mr. Vasconcelos indicated that Standard Methods states that media other than BHI broth may be used.

REFERENCES

APHA, AWWA, and WPCF. 1965. Standard method for the examination of water and wastewater. 12th ed. Am. Pub. Health Assoc., New York 769P.

Hanes, N. B., G. A. Delaney and C. J. O'Leary. 1965. Relationship between Escherichia coli Type I, coliform and enterococci in water. (Presented at a meeting of the Sanitary Section. B.S.C.E, on March 3, 1965)

Kaushik, N. K. and J. K. Bewtra. 1965. Incidence of coliforms and enterococci in natural waters. Environ. Health. 7:32-38.

Litsky, W., W. L. Mallmann and C. W. Fifield. 1955. Comparison of the MPN of E. coli and enterococci in river waters. Am. J. Pub. Health. 45:1049-1053.

Mallmann, W. L. and F. B. Seligmann. 1950. A comparative study of media for the detection of streptococci in water and sewage. Am. J. Pub. Health. 40: 286-289.

Velz, C. J. 1951. Graphical approach to statistics. Water and Sewage Works. 98:66-73.

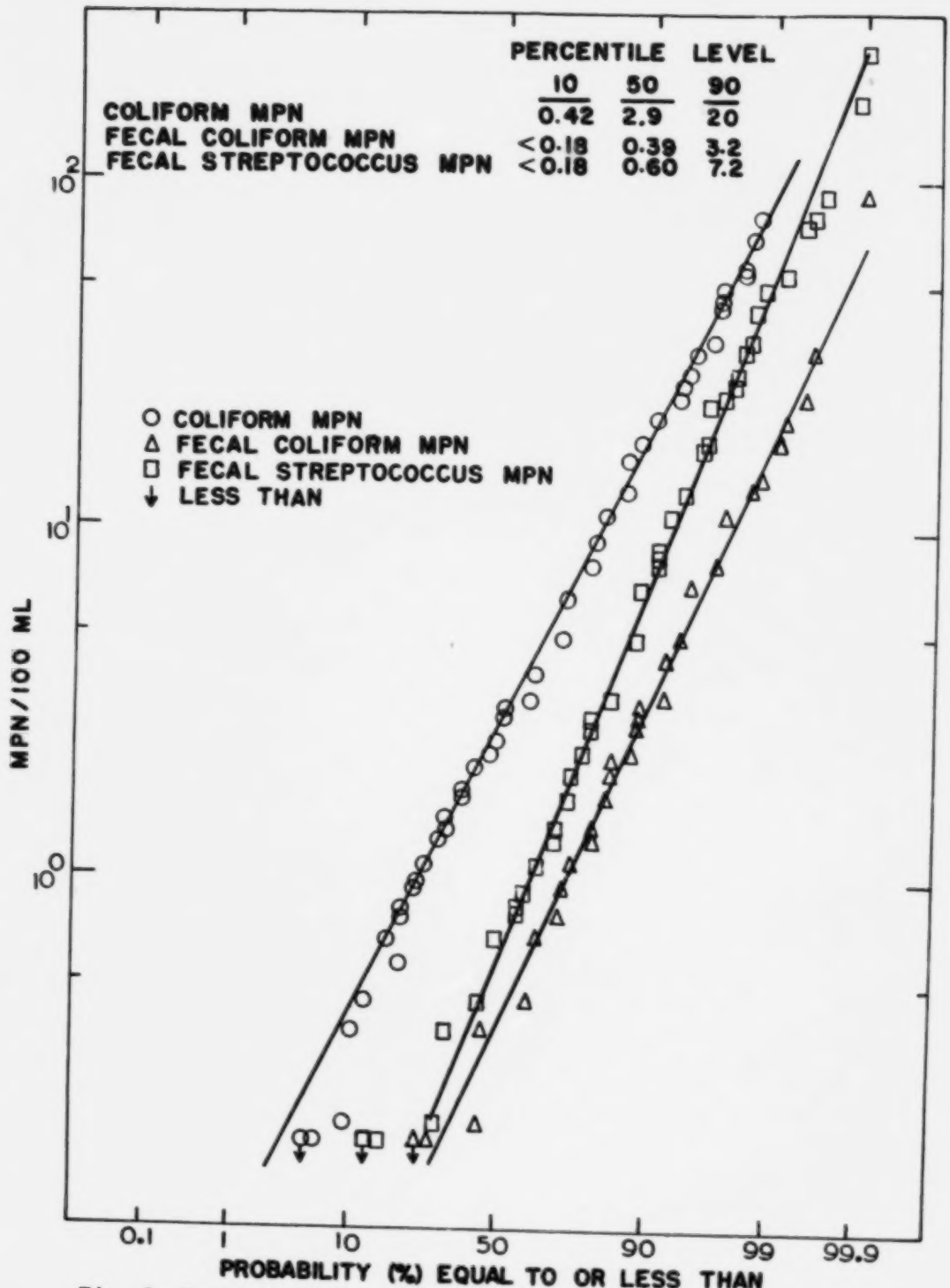

Fig. 1 Comparison of confirmed coliform, fecal coliform and fecal streptococcus MPN's

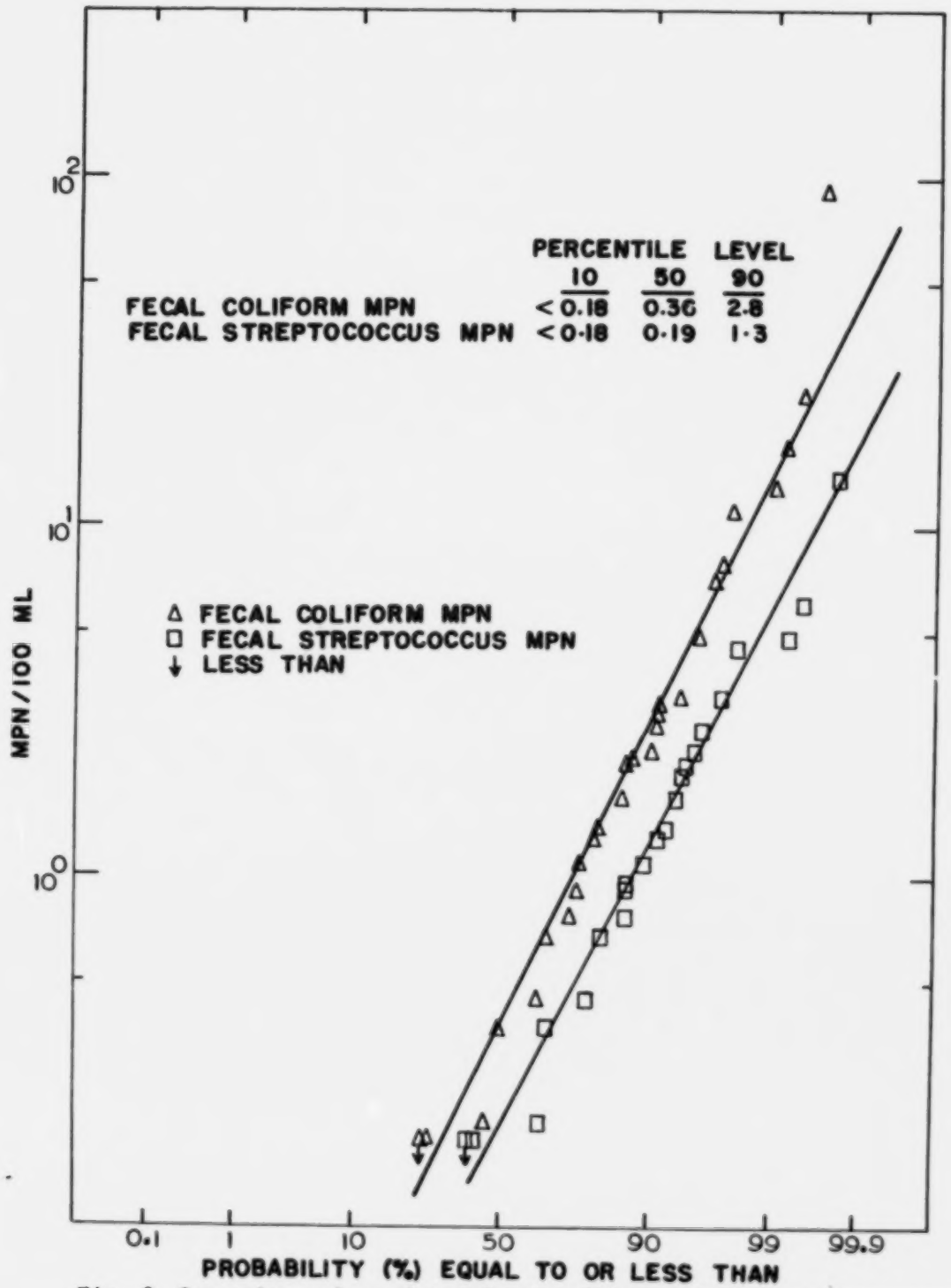

Fig. 2 Comparison of completed fecal coliform and fecal streptococcus MPN's

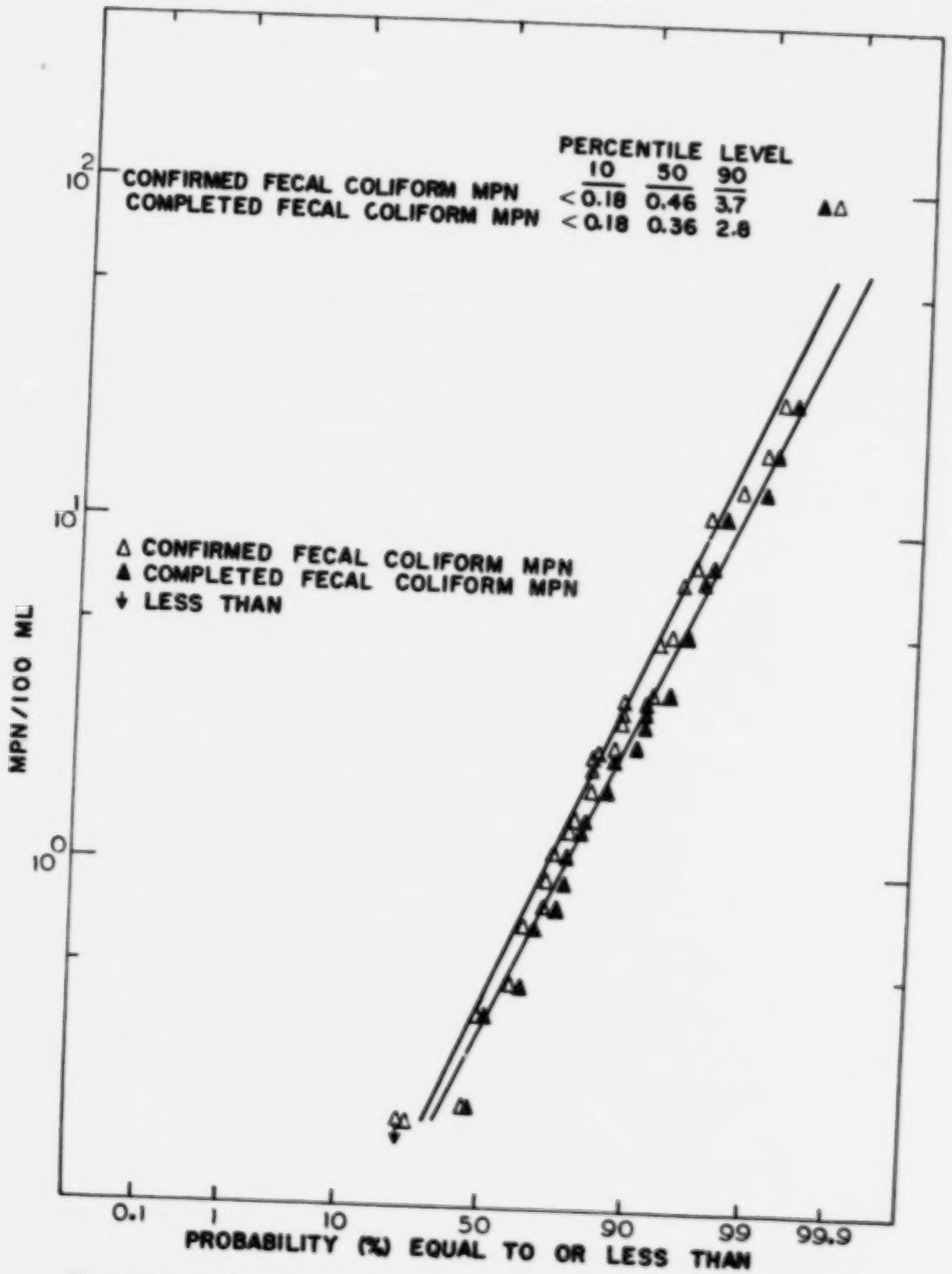

Fig. 3 Comparison of confirmed and completed fecal coliform MPN's

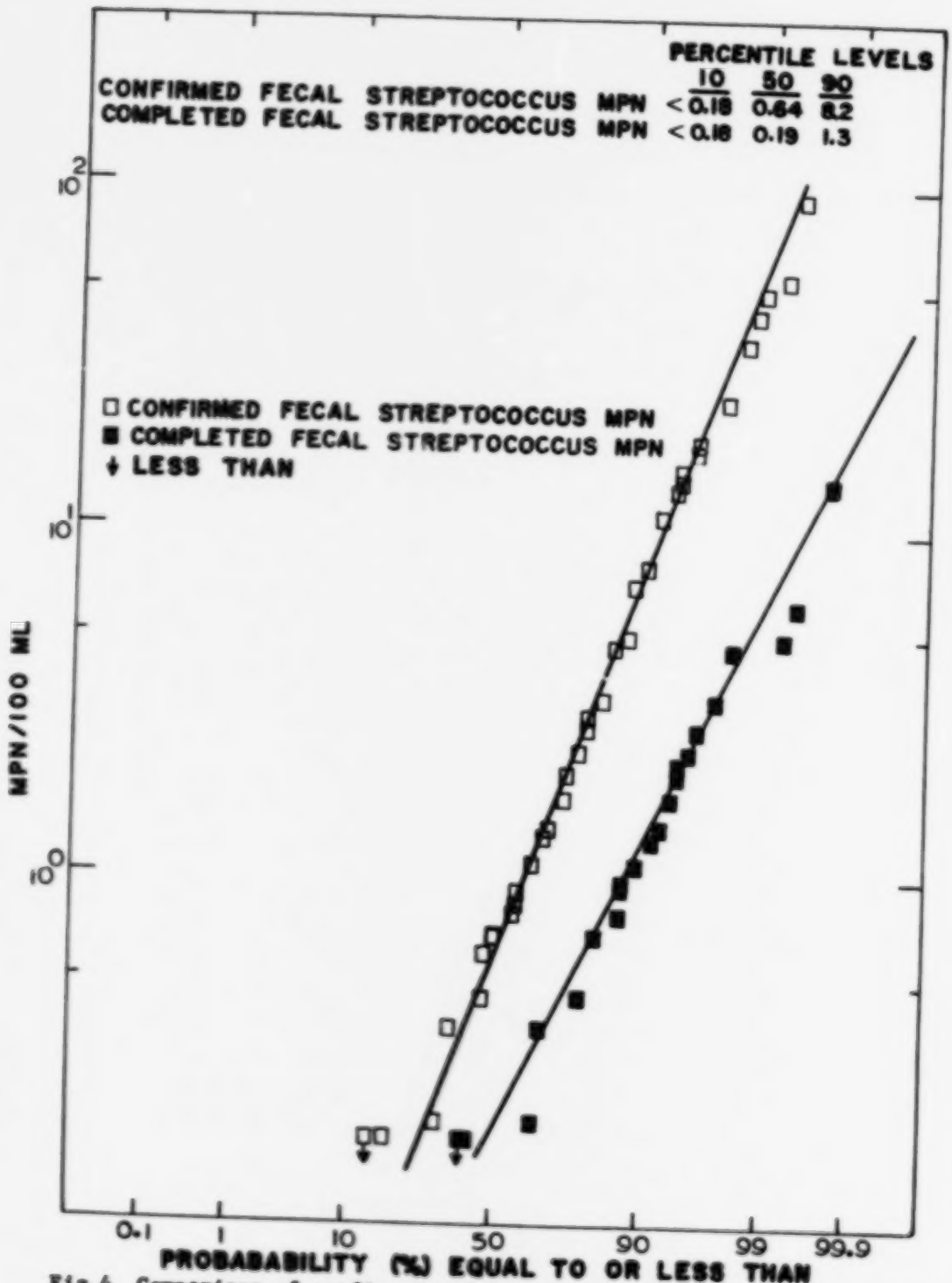

Fig.4 Comparison of confirmed and completed fecal streptococcus MPN's

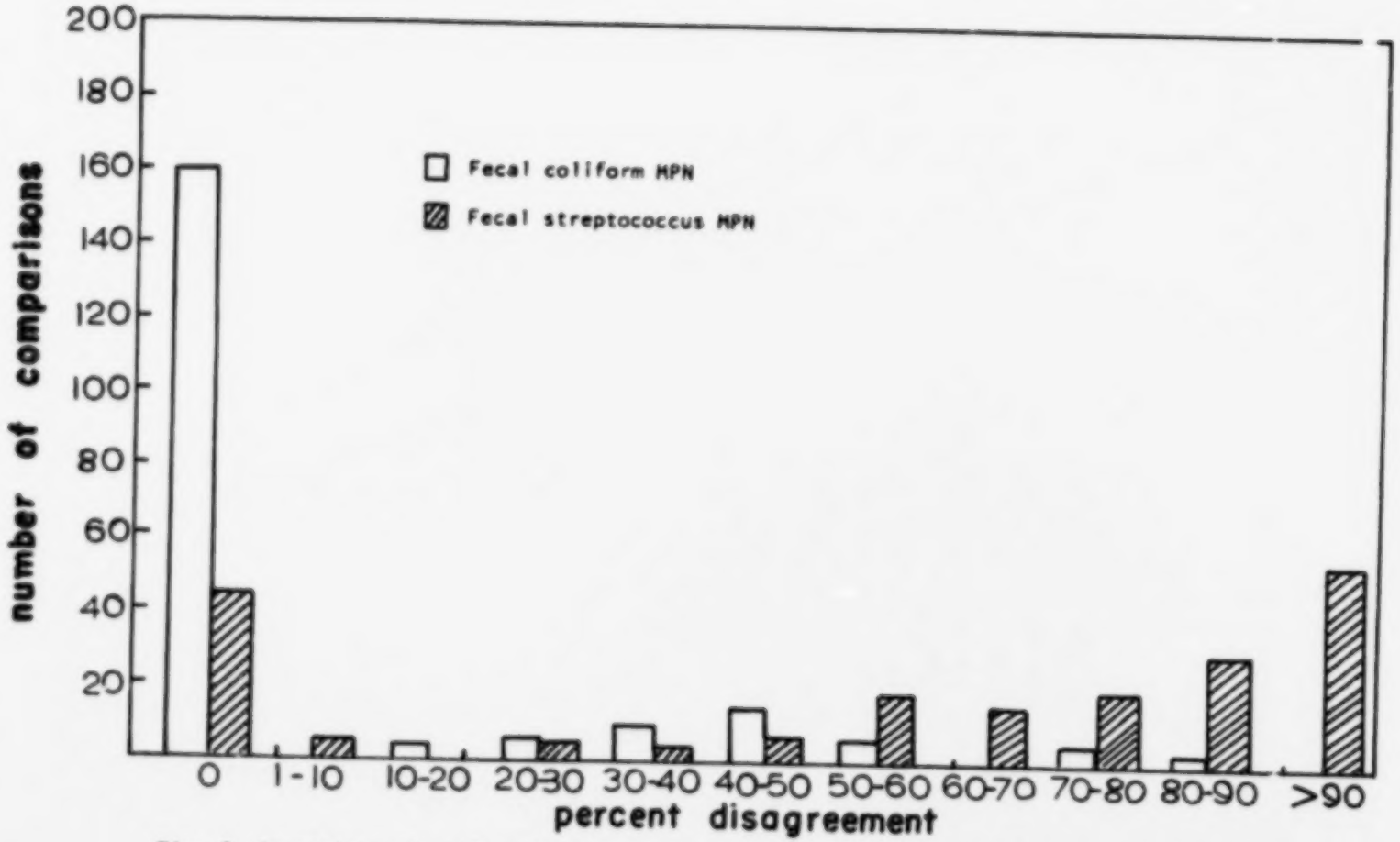

Fig. 5 Comparison of confirmed and completed fecal coliform MPN and fecal streptococcus MPN

Table 1. Data from confirmed coliform, fecal coliform and fecal streptococcus MPN's

Index	No. Determinations	Probability Percentiles 10	50	90	Median	Standard Deviation (Log)	Range
Coliform MPN/100 ml	770	0.42	2.9	20	3.3	0.640	<0.18-170
Fecal coliform MPN/100 ml	755	<0.18	0.39	3.2	0.45	0.700	<0.18-92
Fecal streptococcus MPN/100 ml	760	<0.18	0.60	7.2	0.79	0.840	<0.18-240

Table 2. Data from confirmed and completed fecal coliform and fecal streptococcus MPN's

Index	No. Determinations	Probability Percentiles 10	50	90	Median	Standard Deviation (Log)	Range
Confirmed Fecal Coliform MPN/100 ml	410	<0.18	0.46	3.7	0.45	0.723	<0.18-92
Completed Fecal Coliform MPN/100 ml	395	<0.18	0.36	2.8	0.45	0.689	<0.18-92
Confirmed Fecal Streptococcus MPN/100 ml	428	<0.18	0.64	8.2	0.79	0.856	<0.18-240
Completed Fecal Streptococcus MPN/100 ml	423	<0.18	0.19	1.3	0.20	0.634	<0.18-14

STUDIES ON THE INCIDENCE OF CLOSTRIDIUM BOTULINUM TYPE E IN SOME WEST COAST ESTUARINE AREAS

T. H. Ericksen

INTRODUCTION

This study is a continuation of the investigation reported on at the 1965 Northwest Shellfish Sanitation Research Planning Conference. The presence of C. botulinum Type E in the marine environment and marine products of the Pacific Northwest has been well established by Dolman (1957a), Dolman (1957b), Dolman and Iida (1963), Eklund (1965), and Ericksen and Beck (1965). There exists, therefore, a possible relationship between this organism and marine sea life. This organism has been isolated from fish as reported by Prevot and Huet (1951), Dolman and Chang (1956), Nakamura et al (1956), Johannsen (1963), and Foster et al (1965). Presnell (1966) reported the isolation of this organism from naturally occurring eastern oysters from Mobile Bay, Alabama. Marine mammals (Dolman and Iida, 1963) have also been implicated in botulism outbreaks. Therefore, because of the feeding habits of shellfish and the practice of eating them raw or perhaps only mildly cooked, the incidence of this organism in estuarine areas is of public health significance.

To determine the incidence of C. botulinum Type E in some estuaries of the Pacific Northwest, samples of mud, soil and shellfish were collected and examined from estuarine areas in the states of Washington, Oregon and California.

METHODS AND MATERIALS

Sampling:

Samples of estuarine mud, soil and shellfish were collected from various sites on the West Coast. Samples collected from the State of Washington were collected with the cooperation of the Washington State Department of Health. Samples from Yaquina Bay, Oregon and Humboldt Bay, California were collected by Dr. K. K. Chew, College of Fisheries, University of Washington, Seattle, Washington. Mud, soil and shellfish samples were obtained from the same immediate area where possible. Samples were collected using aseptic technics. The samples were transported to the laboratory in refrigerator cases. Samples were examined immediately upon arrival at the laboratory or frozen at -20 C for examination at a future time.

Sampling Areas:

Samples were obtained from the areas shown in Figures 1, 2 and 3. These areas represented some of the shellfish growing areas on the West Coast. These sites provided a variety of environmental factors. Brief descriptions of the sampling areas are given below.

1. Burley Lagoon: This estuary located at Purdy, Washington is primarily a commercial shellfish growing area. Estuarine mud, Pacific oysters, Native Littleneck and Manila clams were obtained from sites within this estuary. Mud and soil samples from various stations on Burley Creek were also examined.

2. Port Washington Narrows: This narrow waterway leads to Dyes Inlet. This waterway divides the town of Bremerton, Washington. On the north bank of these narrows lies the Mannette Sewage Treatment Plant. Estuarine mud, Native Littleneck clams and sewage influent and effluent were collected.

3. Big Beef Harbor: This inlet is an unpolluted area with some commercial shellfish activity and is located on Hood Canal, northwest of Bremerton, Washington. Mud, Pacific oysters and clams were collected and examined from this area.

4. Yaquina Bay, Oregon: Supporting commercial shellfish activity, this bay is located on the Pacific Coast of Oregon at Newport, Oregon. Pacific and Native oysters, mud and soil were obtained from this area.

5. Humboldt Bay, California: This large estuary lies on the northern coast of California near the town of Eureka, California. This is a major commercial shellfish producing area in California. Mud and Pacific oyster samples were obtained from this area.

Bacteriological Methods:

An outline of the procedures for the bacteriological examination of samples is shown in Fig. 4. The presence of C. botulinum was determined by the methods described by Eklund and Poysky (1965). Some of the samples were also examined for the presence of anaerobic spores. Two methods were used to destroy vegetative cells in the samples. The first method was heat treatment of the sample or dilution in a 60 C water bath for 13 minutes followed by dilution and plating with brain heart infusion (BHI) agar. The duplicate pour plates were then incubated anaerobically for 48 hours at 30 C. The number of spores was then determined by using a Quebec colony counter. The second method

for eliminating vegetative cells included treatment of the sample with 95% ethyl alcohol as described by Johnston, Harmon and Kautter (1964). A known weight of sample was treated with an equal weight of alcohol and allowed to stand for one hour with occasional shaking. After standing, duplicate pour plates were made, then the plates were incubated anaerobically for 48 hours at 30 C. The plates were counted after incubation as previously described.

RESULTS

A summary of the results of the examination of a total of 49 mud, 16 soil and 33 shellfish samples for the presence of C. botulinum is given in Table 1. Only four positive samples were found, all of which were mud samples. One positive mud sample was collected from Burley Lagoon. A positive mud sample had been obtained previously from the same area. The remaining three positive mud samples were obtained from Yaquina Bay, Oregon. These mud samples were obtained in association with oyster samples from the same area. None of the shellfish, including those samples taken adjacent to the positive mud samples, exhibited positive results.

Results of plate counts of those samples undergoing only heat treatment are shown in Table 2. These data indicated at least a tenfold higher count in mud as compared to shellfish samples from the same immediate area. Stream bed mud samples were only slightly higher than those of estuarine mud. The soil samples associated with the stream bed mud samples were tenfold higher than the mud samples.

Results of samples treated with both heat and alcohol are shown in Table 3. Viable spore counts obtained were similar with both methods. Somewhat higher counts were obtained in some instances with the alcohol treatment.

DISCUSSION

Four positive estuarine mud samples were found in two West Coast estuarine areas. No positive results were obtained from shellfish, soil, sewage influent or sewage effluent samples. One of the positive samples was obtained from a site at which a positive sample had been previously obtained. The three remaining positive samples were obtained on two consecutive collecting trips to the same site. Overall, about 12 per cent of the mud samples were positive for C. botulinum Type E. Eklund (1966) found approximately 30 per cent of over 600 mud samples from both fresh water and marine areas on the Pacific Coast were positive

for C. botulinum Type E. Ericksen and Beck (1965) reported approximately 21 per cent of 38 estuarine mud samples were positive for Type E botulism. Dolman (1957b) reported approximately 13 per cent of 47 sea mud samples from British Columbia, Canada were positive. Other ecological studies in Japan (Nakamura et al, 1955), Denmark (Pedersen, 1955), Scandinavia (Johannsen, 1963; Cann et al, 1955), the Great Lakes Region of the United States (Bott et al, 1964), and Greenland (Pedersen, 1955) have indicated a broad distribution of this organism.

As reported at the 1965 Northwest Shellfish Sanitation Laboratory Research Planning Conference, no shellfish samples exhibited positive results, even those shellfish samples collected in association with positive mud samples. Cabelli et al (1965) and Presnell (1966) have reported the presence of C. botulinum in naturally occurring shellfish. Various investigations (Dolman, 1957b; Bott et al, 1964; Nakamura et al, 1956; Prevot and Huet, 1951; Johannsen, 1963) have reported the presence of C. botulinum Type E in fish. Meyer (1950) reported four outbreaks of botulism caused by commercial packs of clams. However, no outbreaks have been reported due to the consumption of fresh shellfish.

Although C. botulinum Type E can be isolated from estuarine and marine areas, the original source of the organisms found in these areas is unknown. Two main possibilities exist. The organism may be a part of the normal flora of these environments. Another possibility is that the organism has a terrigenous origin and merely survives in estuarine and marine areas.

The limited anaerobic spore plate counts indicated soil samples to have the highest counts. Shellfish exhibited the least number of spore formers. Muds contained fewer viable spore formers than soils. This pattern was similar to that reported for aerobic counts for oysters and mud by Vaughn and Jones (1964). The samples treated with heat and alcohol indicated only slightly higher plate counts using the alcohol technic.

This study will be continued by the systematic sampling from one estuarine area in which the presence of C. botulinum Type E has been indicated. This site will be Burley Lagoon at Purdy, Washington. The relationship of various environmental factors such as rainfall, wind direction, use of uplands, etc. to incidence of the organism will be studied. Determination of the origin of the organism as pertains to a continuous or intermittent source will be investigated. Studies on the development of necessary enumeration and isolation technics will also be made.

COMMENTS BY PARTICIPANTS

The discussion centered around the relationship of these results to those obtained by Dr. Eklund in his investigations. Dr. Sparks inquired as to whether this organism is of marine or terrestrial origin. Mr. Ericksen indicated that there is some evidence to support either of these viewpoints.

REFERENCES

Bott, T. L., J. S. Deffner, E. M. Foster and E. McCoy. 1964. Ecology of C. botulinum in the Great Lakes. Botulism. PHS Pub. No. 999-FP-1.

Cabelli, V. J., I. Anderson, N. L. Richards, W. P. Heffernan, and G. P. Hoskins. 1965. Progress Report: Clostridium botulinum in the estuarine environment. Northeast Shellfish Sanitation Research Center, Narragansett, R. I.

Cann, D. C., B. B. Wilson, G. Hobbs and J. M. Shewan. 1965. The incidence of Clostridium botulinum Type E in fish and bottom deposits in the North Sea and off the coast of Scandinavia. J. Appl. Bacteriol. 28:426-430.

Dolman, C. E. and H. Chang. 1953. The epidemiology and pathogenesis of Type E fish-borne botulism. Can. J. Pub. Health. 44:231-244.

Dolman, C. E. 1957a. Recent observations on Type E botulism. Can. J. Pub. Health. 48:187-198.

Dolman, C. E. 1957b. Type E (fish-borne) botulism: A review. Jap. J. Med. Sci. Biol. 10:383-395.

Dolman, C. E. and H. Iida. 1963. Type E botulism: Its epidemiology, prevention and specific treatment. Can. J. Pub. Health. 54:293-308.

Eklund, M. W. 1965. Incidence of Clostridium botulinum on the Pacific Coast of the United States. Proc. Northwest Shellfish Sanitation Research Planning Conference. PHS Pub. No. 999-FP-6.

Eklund, M. W. and F. Poysky. 1965. Clostridium botulinum Type F from marine sediments. Science. 149:306.

Eklund, M. W. 1966. Personal Communication.

Ericksen, T. H. and W. J. Beck. 1965. Ecological studies for presence of toxin-producing, Gram positive, spore forming bacteria. Proc. Northwest Shellfish Sanitation Research Planning Conference. PHS Pub. No. 999-FP-6.

Foster, E. M., J. S. Deffner, T. L. Bott and E. McCoy. 1965. Clostridium botulinum food poisoning. J. Milk Food Technol. 28:81-91.

Johannsen, A. 1963. Clostridium botulinum in Sweden and adjacent waters J. Appl. Bacteriol. 26:43-47.

Johnston, R., S. Harmon and D. Kautter. 1964. Method to facilitate the isolation of Clostridium botulinum Type E. J. Bacteriol. 88:1521-1522.

Nakamura, Y., H. Iida, K. Saiki, K. Kanazawa and T. Karashimada. 1956. Type E botulism in Hokkaido, Japan. Jap. J. Med. Sci. and Biol., 9:45-58.

Pedersen, H. O. 1955. On Type E botulism. J. Appl. Bacteriol. 18:619-628.

Presnell, M. W. 1966. Unpublished data.

Prevot, A. R. and M. Huet. 1951. Esistence en France du botulisme humain d origine pisciaire et de Cl. botulinum E. Bull. Acad. Nat. Med., Nos. 25 and 26:432-434.

Vaughn, M. W. and A. W. Jones. 1964. Bacteriological survey of an oyster bed in Tangier Sound, Maryland. Chesapeake Science 5:167-171.

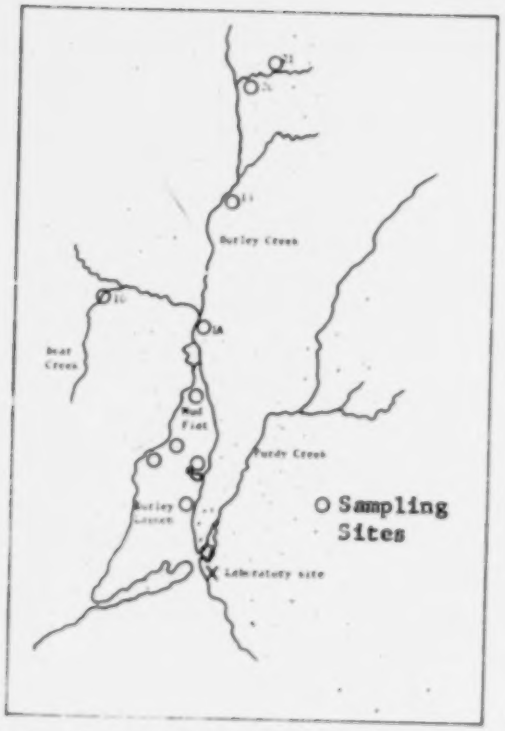

Fig. 1 Sampling sites in Burley Lagoon and its watershed

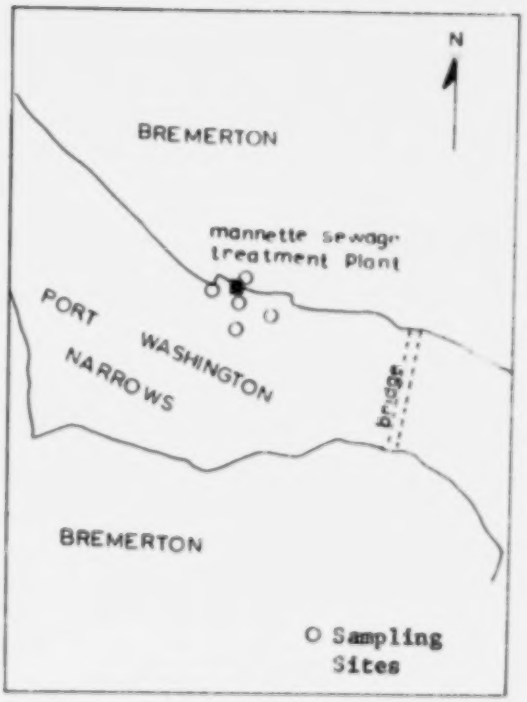

Fig. 2 Sampling sites at the Mannette Treatment Plant

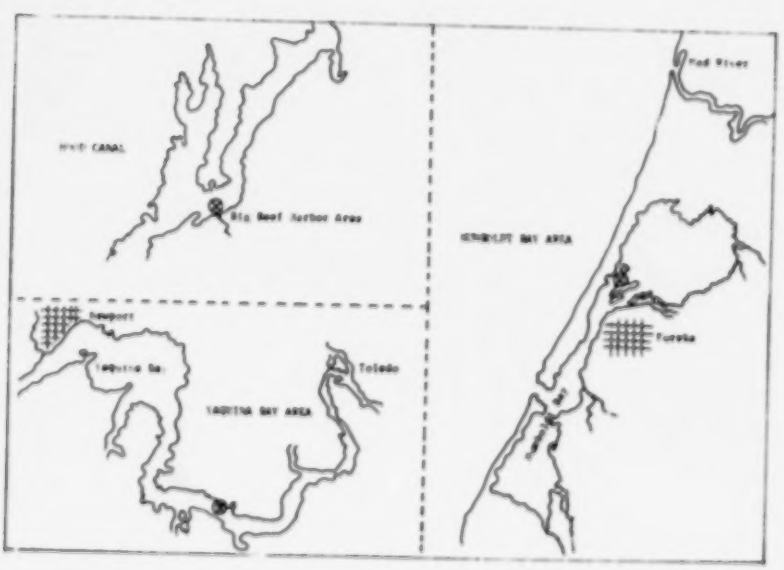

Fig. 3 Other sampling areas in Washington, Oregon and California

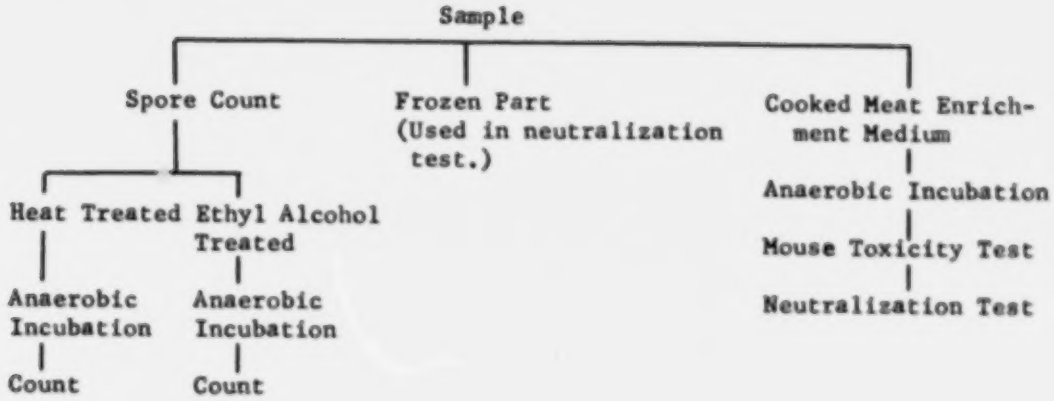

Fig. 4 Procedures for the bacteriological examination for presence of C. botulinum in mud, soil and shellfish samples

Table 1. Results of mouse toxicity tests on mud, soil and shellfish samples from some West Coast estuarine areas

	Area									
	Burley Lagoon		Port Washington Narrows		Big Beef Harbor		Yaquina Bay		Humboldt Bay	
	No. Tested	No. Positive	No. Tested	No. Positive	No. Tested	No. Positive	No. Tested	No. Positive	No. Tested	No. Positive
Estuarine mud	17	1	2	0	2	0	8	3	5	0
Stream bed mud	11	0								
Terrestrial soil	14	0								
Sewage influent			2	0			2	0		
Sewage effluent			2	0						
Pacific oyster	6	0			1	0	5	0	5	0
Olympia oyster							3	0		
Manila clam	6	0			1	0				
Native Littleneck clam	4	0	2	0	—	—	—	—	—	—
TOTAL	58	1	8	0	4	0	18	3	10	0

Table 2. Geometric mean values of samples examined for viable spores by heat treatment and subsequent plating

Sample	No. of Samples	Plate Count/g
Pacific oysters	4	160
Adjacent mud	4	3,000
Manila clams	4	780
Adjacent mud	4	3,700
Native Littleneck clams	2	67
Adjacent mud	2	4,200
Soil[a]	5	49,000
Mud[b]	5	4,900

[a]Soil collected adjacent to the stream.
[b]Mud collected from the stream bed.

Table 3. Comparison of viable spore counts of heat and alcohol treated samples

Sample	Plate count/g	
	Heat Treated	Alcohol Treated
Pacific oysters	50	60
Adjacent mud	680	870
Pacific oysters	30	70
Adjacent mud	590	370
Native Littleneck clams	90	90
Adjacent mud	630	1,500
Native Littleneck clams	90	160
Adjacent mud	660	1,400
Sewage influent	1,500	1,600
Sewage effluent	70	100

ACCUMULATION AND RETENTION OF *CLOSTRIDIUM BOTULINUM* TYPE E SPORES BY MANILA CLAMS

T. H. Ericksen

INTRODUCTION

Clostridium botulinum Type E has been found in the marine environment of the Pacific Northwest as reported by Dolman (1957a), Dolman (1957b), Eklund (1965), and Ericksen and Beck (1965). Although cases of botulism associated with the consumption of fresh shellfish have not been reported, the possibility of contact between shellfish and *C. botulinum* Type E exists. Reports by Cabelli *et al* (1965) and Presnell (1966) have indicated that *C. botulinum* may be found in naturally occurring shellfish. Because shellfish are eaten raw or in a mildly cooked manner, which may not destroy the organism or its toxin, it is of public health significance to determine the fate of the organism in shellfish. It is also desirable to study the factors which may induce outgrowth of *C. botulinum* Type E spores, and possible toxin production during storage of shellfish contaminated by these bacteria.

METHODS AND MATERIALS

Spore Suspension:

A culture of the Beluga strain of *C. botulinum* Type E was obtained from Dr. Melvin W. Eklund, Bureau of Commercial Fisheries, Seattle, Washington. The spore suspension was prepared in trypticase-peptone-glucose (T-P-G) medium (Schmidt *et al*, 1962). After several passages through T-P-G medium the final inoculated medium was centrifuged under refrigeration, washed and then heated for 13 minutes in a 60 C water bath. The number of viable spores was then determined using the pour plate method with brain heart infusion (BHI) agar. The plates were inoculated in duplicate and incubated anaerobically at 30 C for 48 hours. Anaerobic conditions were obtained by replacement of the atmosphere in a #3 Case Anaero jar with a gaseous mixture of 95% nitrogen and 5% carbon dioxide. The spore suspension was kept frozen until used.

Shellfish:

The shellfish used in this study were Manila clams (*Tapes philippinarum*). Shellfish were collected from a site near the laboratory. Approximately 300 clams were collected for each experiment. The clams were washed to remove mud and fouling debris and then were placed in the pollution flat. A control sample of shellfish was examined prior to addition of the clams to the pollution flat.

Accumulation:

The accumulation phase of this study was conducted in an isolated, closed system, which consisted of a wooden flat (113x59.7x25.4 cm). The flat was equipped with an aeration device which also circulated the overlying seawater. Sufficient spore suspension to give 100 spores/ml of seawater was added to the pollution flat containing 100 liters of U.V. treated seawater and 300 clams. The shellfish remained in this flat for a 48 hour period. This period was modified to 24 hours for the last experiment. The siphons of the clams were observed to be extended during the accumulation phase of the three experiments.

Storage:

After the accumulation phase, the clams were removed from the pollution tank and surface debris was removed from the shells. The clams were then divided into three groups and stored in plastic sacks in closed containers at 20 C, 10 C and -20 C.

Sampling:

The seawater and clams were sampled immediately before addition of the spore suspension and at 12 hour intervals for the remainder of the accumulation phase. During the accumulation phase of experiment three, U. V. treated seawater in a stoppered flask was placed in the pollution flat and viable spore and 20 C plate counts were made at 12 and 24 hours. Shellfish samples were selected at random and consisted of ten clams per sample. Seawater samples consisted of a pool of ten random 10 ml portions.

Shellfish stored at 20 C, 10 C and -20 C were sampled according to the following schedule:

1. 20 C
   0, 2, 4 and 7 days

2. 10 C
   0, 2, 4, 7, 10 and 15 days

3. -20 C
   0, 1, 3, 6, 9 and 12 months.

"Gapers" at each temperature were removed at each sampling interval and examined.

Bacteriological Methods:

The bacteriological examination of shellfish and seawater samples included 20 C aerobic plate counts using MacLeod's seawater medium (MacLeod et al, 1954), anaerobic viable spore plate counts using SPI agar for experiment one, T-P-G medium (Schmidt et al, 1962) with 2

percent agar added for experiment two, and reinforced clostridial medium (RCM) for experiment three. The change in plating media was made because data obtained from investigations with known spore suspensions indicated that each successive medium gave more precise results. Mouse toxicity and neutralization test methods used were those described by Eklund and Poysky (1965). Toxicity tests were carried out directly on seawater and clam samples at each sampling interval. Toxicity tests were also performed on portions of the samples incubated anaerobically at 25 C for 5 days in cooked meat enrichment medium (Dolman, 1957). This medium was modified by using a dehydrated cooked meat medium as the basic ingredient. Neutralization tests were performed with botulinal antitoxins obtained from the Communicable Disease Center, Atlanta, Georgia.

Safety Precautions:

Laboratory personnel have received immunization with the pentavalent botulism toxoid. The accumulation flat was enclosed in a plastic curtain and isolated from the rest of the laboratory. Immediately after the accumulation phase, 100 ppm chlorine was added to the polluted seawater. As soon as possible the water was autoclaved for one hour before disposal. The flat was then mopped with a chlorine solution. Ten random swabs of 16 square inches each were taken from the inner surface of the pollution flat. These swabs were incubated anaerobically in cooked meat enrichment medium and mouse toxicity tests were performed. No positive tests were observed.

RESULTS

The accumulation phase results of the three experiments are shown in Tables 1 and 2. The viable spore counts shown in Table 1 remained relatively unchanged. However, spore counts of the clams were higher than those of the seawater at each sampling interval. The spore counts for seawater ranged from 5/ml to 400/ml. Spore count levels in the seawater were considerably lower than the expected 100 spores/ml. The 20 C aerobic plate count results shown in Table 1 indicate no distinct pattern of change in the clams. However, 20 C plate counts on the seawater indicated an increase. Again, the 20 C plate counts were generally higher in clams than in the seawater. The results in Table 2 show that toxicity tests made directly on seawater and clams during the pollution phase were all negative. Mouse toxicity and neutralization tests on portions of seawater samples incubated anaerobically in cooked meat enrichment medium were positive at 48 hours in experiment one, and at 12 and 24 hours in experiments two and three. No distinct pattern was indicated. All portions of exposed shellfish samples anaerobically incubated in cooked meat medium exhibited positive toxicity tests.

The storage phase results from the three experiments are shown in Table 3 and 4. The viable spore count and 20 C plate count data are shown in Table 3. The viable spore counts of shellfish stored at 10 C

and 20 C exhibited little change as storage progressed. This pattern was also exhibited by samples of "gapers" at these two temperatures. The 20 C aerobic plate counts increased at both 10 C and 20 C during storage. The 20 C plate counts of clams stored at 20 C increased at a faster rate than those stored at 10 C. This pattern was expected and the plate counts at both temperatures followed patterns found in previous Manila clam shellstock storage studies (Ericksen, Hoff and Beck, 1964). Results of toxicity tests on stored clams are shown in Table 4. All toxicity tests carried out directly on stored clam samples were negative.

With two exceptions, mouse toxicity and neutralization tests carried out on incubated portions of the stored shellfish indicated the presence of C. botulinum Type E in exposed shellfish stored at 10 C and 20 C respectively.

In one instance a sample of sediment containing clam feces and seawater was collected from the bottom of the pollution flat. A modified method for determining numbers of organisms was carried out separately on the sediment and the seawater associated with it. The sediment and associated seawater were separated and dilutions were made. Portions of the various dilutions of the sediment and seawater were inoculated into tubes of cooked meat enrichment medium. Toxicity tests on dilutions exhibiting growth indicated that the sediment had positive results at a tenfold higher dilution than the seawater. The higher values in the sediment may have been due to the organism being in the feces of the clams or possibly to natural sedimentation of the spores from the overlying seawater.

DISCUSSION

These experiments indicate that clams exposed to seawater containing spores of C. botulinum Type E in a closed system may accumulate the spores. Although toxin could not be detected upon direct examination of shellfish stored at 10 C and 20 C, positive results were obtained from those portions of the samples incubated anaerobically in cooked meat enrichment medium. Therefore, these data indicate that C. botulinum Type E spores present in the clams remained viable in the shellfish when stored at 10 C and 20 C. This would also indicate that the factors necessary for outgrowth and toxin production were not present. Graikoski and Kempe (1963) reported maximum toxin titers to occur at 19 and 13.7 C in fish and seafood extracts. Shewan and Cann (1965) have reported toxin production in vacuum packed herring, kippers and smoked haddock stored at 20 C to be present at 1, 2 and 3 days respectively. The same investigations reported toxin production in herring, kippers, cod and plaice stored at 10 C occurred at 5, 9, 10 and 10 days respectively.

The erratic viable spore count data obtained from these experiments, particularly the contaminated seawater samples, indicates a necessity for improved methods for quantitative enumeration of C. botulinum Type E spores from seawater and shellfish. The data on toxicity tests of incubated seawater also emphasize a need for further investigation on enumeration and

isolation methods. The sporadic positive toxicity tests on incubated seawater compared to the relatively uniform results of incubated clam samples also indicates further studies on factors affecting toxin production should be made.

Future study will include pollution of Manila clams and other species of West Coast shellfish. Emphasis will also be placed on development of methods for the quantitative recovery and enumeration of C. botulinum spores from seawater and shellfish. Initial experiments will include artificial pollution of seawater with known quantities of spores and development of media for the optimum recovery of these spores. These media will be developed on a cooperative basis with other shellfish sanitation centers to find essential constituents necessary for outgrowth of spores. These methods will be tested on naturally occurring samples. Investigation of the factors necessary to induce toxin production during accumulation and storage will be continued. Investigation will also be carried out on effects of dry storage on shucked shellfish which have been exposed to contamination as shellstock.

COMMENTS BY PARTICIPANTS

Mr. Dupuy asked whether or not feces and pseudofeces were examined separately. Ericksen answered that the deposits examined consisted of material deposited on the bottom of the tank. Mr. Neufeld asked about methods used for anaerobic incubation and whether or not moisture was a problem. Mr. Ericksen answered that both Brewer and Case anaerobic jars were used and that gas packs were used with the Brewer jars. Moisture problems were avoided by placing paper towels in the bottom of the jars to absorb condensation.

REFERENCES

Dolman, C. E. 1957a. Recent observations on Type E botulism. Can. J. Pub. Health. 48:187-198.

Dolman, C. E. 1957b. Type E (fish-borne) botulism: A review. Jap. J. Med. Sci. Biol. 10(b):383-395.

Eklund, M. W. 1965. Incidence of Clostridium botulinum on the Pacific Coast of the United States. Proc. Northwest Shellfish Sanitation Research Planning Conference. PHS Pub. No. 999-FP-6.

Eklund, M. W. and F. Poysky. 1965. Clostridium botulinum Type F from marine sediments. Science, 149(3681):306.

Ericksen, T. H., and W. J. Beck. 1965. Studies on the incidence of Clostridium botulinum Type E in some West Coast estuarine areas. Proc. Northwest Shellfish Sanitation Research Planning Conference. PHS Pub. No. 999-FP-6.

Graikoski, J. F., and L. L. Kempe. 1963. Factors influencing the toxin production by Clostridium botulinum Type E. Bacteriol. Proc. 1963:8.

MacLeod, R. A., E. Onofrey, and M. E. Norris. 1954. Nutrition and metabolism of marine bacteria. I. Survey of nutritional requirements. J. Bacteriol. 68:680-686.

Presnell, M. W. 1966. Unpublished data.

Schmidt, D. F., W. K. Nank, and R. V. Lechowich. 1962. Radiation sterilization of food. II. Some aspects of the growth, sporulation and radiation resistance of spores of Clostridium botulinum Type E. J. Food Sci., 27(1):77-81.

Shewan, J. M., and D. C. Cann. 1965. Botulism and Fishery Products. Torry Research Station. Torry Advisory Note No. 22.

Table 1. Accumulation of *Clostridium botulinum* Type E spores by Manila clams, *Tapes philippinarum*
Plate Counts

Time in hrs	Spore Count/ml or g						20 C Plate Count/ml or g					
	Water			Clams			Water			Clams		
	Exp.			Exp.			Exp.			Exp.		
	1	2	3	1	2	3	1	2	3	1	2	3
0	<1	<1	35	240	120	200	35	320	1,400	84,000	75,000	21,000
12	40	8	10	320	270	80	110	100	100,000	1,400	9,100	46,000
24	<1	11	15	330	140	5	110	460	19,000	16,000	19,000	30,000
36	10	2		200	400		150	2,200		7,500	110,000	
48	35	5		270	390		6,700	2,900		29,000	66,000	
Control												
12		10							16,000			
24		5							9,200			

Table 2. Accumulation of *Clostridium botulinum* Type E spores by Manila clams, *Tapes philippinarum*
Toxicity Tests

Time in hrs	Toxicity — No Incubation — Water — Experiment 1	2	3	Clams — Experiment 1	2	3	Toxicity — Samples Incubated — Water — Experiment 1	2	3	Clams — Experiment 1	2	3
0	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Lab. Acc.	Neg.
12	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Pos.	Pos.	Pos.	Pos.	Pos.
24	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Pos.	Pos.	Pos.	Pos.	Pos.
36	Neg.	Neg.	-	Neg.	Neg.	-	Neg.	Neg.	-	Pos.	Pos.	-
48	Neg.	Neg.	-	Neg.	Neg.	-	Pos.	Neg.	-	Pos.	Pos.	-

Table 3. Storage of Manila clams, Tapes philippinarum, contaminated with Type E Clostridium botulinum spores
Plate Counts

Storage time in days	Spore Count Experiment 1	2	3	20 C Plate Count Experiment 1	2	3
10 C Storage						
2	110	170	40	50,000	30,000	130,000
4	120	220	190	700,000	100,000	160,000
7	30	150	25	3,000,000	360,000	320,000
10	-	130	-	-	12,000,000	-
15	-	220	-	-	11,000,000	-
20 C Storage						
2	130	130	15	900,000	7,000,000	1,300,000
4	20	140	30	-	12,000,000	8,800,000
Gapers						
10 C Storage						
7	70	-	15	4,600,000	-	4,700,000
10	60	-	<1	610,000	-	4,500,000
15	-	140	-	-	13,000,000	
20 C Storage						
4	-	130	-	-	39,000,000	3,800,000
7	40	110	-	4,600,000	600,000	11,000,000

Table 4. Storage of Manila clams, Tapes philippinarum, contaminated with Type E Clostridium botulinum spores
Toxicity Tests

Storage time in days	Toxicity No Incubation Experiment 1	2	3	Toxicity Incubated Samples Experiment 1	2	3
10 C Storage						
2	Neg.	Neg.	Neg.	Pos.	Pos.	Pos.
4	Neg.	Neg.	Neg.	Neg.	Pos.	Pos.
7	Neg	Neg.	Neg.	Pos.	Pos.	Pos.
10	-	Neg.	-	-	Pos.	-
15	-	Neg.	-	-	Pos.	-
20 C Storage						
2	Neg.	Neg.	Neg.	Pos.	Pos.	Pos.
4	Neg.	Neg.	Neg.	Pos.	Pos.	Pos.
Gapers						
10 C Storage						
7	Neg.	-	Neg.	Pos.	-	Pos.
10	Neg.	-	Neg.	Pos.	-	Pos.
15	-	Neg.	-	-	Pos.	-
20 C Storage						
4	-	Neg.	Neg.	-	Pos.	Neg.
7	Neg.	Neg.	Neg.	Pos.	Pos.	Neg.

STUDIES ON WET STORAGE OF OYSTER AND CLAM SHELLSTOCK

W. Jakubowski and G. J. Vasconcelos

INTRODUCTION

In some commercial operations shellstock is stored in floats or in shallow beds at higher tidal elevations. Depending on float location, tidal conditions and the shucking schedule of the company, shellfish stored in this manner may be partially or completely submerged for several days.

Little work has been done in determining the effect of wet storage on the sanitary quality of West Coast shellfish. Moreover, the effects of water bacterial density, shellfish load, water temperature, salinity and seasonal factors on the accumulation and elimination of bacteria by shellfish in wet storage are not known.

This study was initiated to determine the relative changes in coliform MPN, fecal coliform MPN and plate count of Pacific oysters (Crassostrea gigas) and Manila clams (Tapes philippinarum) subjected to wet storage conditions on a small scale. Results presented in this paper are based on four runs completed during the winter months of 1965-66 and one run performed in July 1966.

MATERIALS AND METHODS

Float:

A diagram of the float used in this study is shown in Fig. 1. Styrofoam-filled 3/4" marine plywood boxes 39" long x 29" wide x 24" deep were used for flotation. A stabilizer, consisting of a 12' long 2x4 with a styrofoam-filled plywood box 18" long x 18" wide x 12" deep on each end, was attached to one end of the float. Galvanized 5/8" wire rope clips were bolted to the frame for supporting the baskets. The entire float was coated with an epoxy resin.

Baskets:

The float contained four baskets, each measuring 36" long x 17" wide x 7" deep. Basket frames were constructed of 5/8" marine plywood and were covered with 1/2" mesh .081 gauge expanded aluminum. One basket, the clam basket, was divided into three compartments by 1/2" mesh .081 gauge expanded aluminum. Galvanized 7/16" wire rope clips

were attached to the ends of the baskets. All parts were given one coat of epoxy resin and one coat of asphaltum varnish. The baskets were suspended about 18" from the top and 12" from the bottom of the float by 1/4" diameter polyethylene rope.

Location of Float:

The float was anchored at each end over a commercial oyster bed in Burley Lagoon southeast of the power line towers and about 150 feet from the sandspit road (Fig. 2). The long axis of the float was oriented in an east-west direction. The baskets were out of the water at about the +6.5' tide level (corrected for Wauna). The float was exposed at about the +5.5' tide level.

Shellfish:

Pacific oysters (Crassostrea gigas) were harvested within a 25' radius of the float. Oysters were picked from the bed and placed directly into the baskets. If the degree of encrustation warranted debarnacling, this was done prior to placing the oysters in the baskets. Each of three baskets contained about 150 oysters. Manila clams (Tapes philippinarum) were harvested from the area indicated in Fig. 2. The clams were rinsed in fresh water and approximately 600 were placed in one basket.

Sampling Schedule:

Shellfish samples consisting of 10 Pacific oysters and 15 Manila clams were examined during two complete tide cycles (a tide cycle consisting of a lower low, higher high, higher low and lower high tide in approximately a 24-hour period). The sampling schedule was initiated on the lower of the daily low tides. Duplicate random oyster, clam and water samples were collected at the low, mid and high points of the first tide cycle and at the low and high points of the second tide cycle. In the fifth experiment, mid tide samples were taken throughout the sampling period. This was done because of the rapid shellfish accumulation-elimination rate observed in preceding experiments.

Test Procedures:

Coliform MPN and fecal coliform MPN determinations on shellfish and seawater were made according to recommended procedures (APHA, 1962). MacLeod's seawater agar was used for plate counts (MacLeod, 1954), with incubation at 20 C for 5 days. During initial float runs, salinity

determinations were made using the hydrometric method of Zerbe and Taylor (1953). In later experiments salinity and temperature were determined with an electrodeless conductive portable salinometer (Industrial Instruments, Inc., Model RS 5-2).

RESULTS

The coliform MPN's fecal coliform MPN's and plate count results of the seawater samples from the first float run are shown in Fig. 3. Seawater and shellfish results compared by individual bacterial index are shown in Figs. 4, 5 and 6.

In this first run samples were taken on an hourly basis, that is, every 2, 3 or 6 hours. The sampling time, therefore, did not always coincide with the low, mid and high stages of the tide. Seawater samples were not taken at low tide when the float was dry but shellfish samples were examined. In succeeding runs seawater and shellfish samples were taken at the low, mid and high points of the tide. Seawater samples also were taken when the float was exposed at low tide.

A comparison of results from multiple seawater and shellfish samples from different positions within the float during the first run indicated no variance in coliform MPN or fecal coliform MPN according to the limits of the test. The Manila clam coliform MPN's and fecal coliform MPN's were slightly higher than those in the Pacific oysters. However, the Pacific oyster plate counts were higher than those in the clams. In general, the density of each index varied inversely with the salinity observations and the predicted height of the tide.

Fig. 7 shows the results of seawater coliform MPN's, fecal coliform MPN's and plate counts for the second experiment and these are compared to shellfish results in Figs. 8, 9 and 10. The bacteriological levels in this experiment generally varied inversely with salinity measurements but the salinity did not vary directly with the predicted height of the tide. Manila clam coliform MPN's, fecal coliform MPN's and plate counts were higher than those in the oysters.

The results of seawater coliform MPN's, fecal coliform MPN's and plate counts for the third and fourth experiments are shown in Figs. 11 and 12 respectively. Bacterial levels here also varied inversely with salinity although the salinity did not vary directly with the predicted tide. A comparison of seawater and shellfish results for the third and fourth experiments is not shown since these followed the same pattern as had been observed in the second experiment.

The seawater bacteriological results of the fifth experiment (the first of the summer series)are shown in Fig. 13. The coliform MPN's and fecal coliform MPN's of the seawater were lower in this experiment than any preceding experiment. However, the plate count determinations were higher than in any previous run. Extremely large and rapid changes in coliform and fecal coliform levels also occurred during this experiment but plate count levels were quite stable. The unusual pattern of changes shown by coliform and fecal coliform levels did not appear to correlate directly with tidal changes.

Comparisons of seawater and shellfish results by bacteriological index are shown in Figs. 14, 15 and 16. Changes in coliform and fecal coliform levels in the shellfish were similar to those in the water. However, it is apparent that changes in the shellfish MPN's were not as great as those in the water. Considerable lag in both accumulation and elimination of these groups is indicated.

Water temperature, salinity and rainfall data for each run are shown in Table 1. Temperature fluctuations were less than 2 C in any of the first three runs, 5 C in the fourth and 8 C in the fifth run. Salinity observations differed widely from one run to the next. Within individual runs the smallest salinity range, about 1.5 o/oo, occurred during the fifth float run and the largest range, about 10 o/oo, occurred during the second and fourth float runs. Rainfall ranged from zero during the fifth experiment to a maximum of 3.8 inches during the second experiment.

DISCUSSION

In general, the highest bacteriological results for both seawater and shellfish samples in all float runs were obtained during the periods of lowest salinity. Conversely, the lowest results were obtained at high salinity readings. The salinity observations, however, did not fluctuate rhythmically with the tide.

A hydrographic study of the lagoon (Kelley, 1964) showed that a large, strong eddy existed in the float area (Fig. 2). Because of this eddy the current always flows south along the west shore of the southern quarter of the lagoon and east along the north shore of the sand spit. Therefore, the water is always flowing in the same direction in the float vicinity on both the ebb and flood tides. The presence of this eddy may explain the lack of correlation between predicted tide heights and observed salinities.

In general, the coliform and fecal colifrom MPN's in seawater and shellfish were influenced mostly by changes in salinity and rainfall. Heavy rainfall and subsequent runoff resulted in higher concentrations of coliform and fecal coliform organisms in the seawater surrounding the float. Shellfish collected from the float showed rapid accumulation-elimination responses to changes in the seawater coliform and fecal coliform content. However, these hydrographic and climatological factors had a lesser influence on changes in the 20 C plate count. While coliform and fecal coliform MPN in shellfish and water fluctuated with changes in salinity and rainfall, plate counts remained more stable. The persistence of these microorganisms enumerated by the 20 C plate count would seem to indicate the presence of natural flora associated with shellfish and their surrounding water.

COMMENTS BY PARTICIPANTS

Drs. Sparks and Dollar inquired about the location of water sampling during several tide stages. Mr. Neufeld asked how indeterminately low values were used. Mr. Vasconcelos replied that they were plotted on probability graph paper in conjunction with determinate values.

Mr. Bower and Dr. Sparks discussed the location of the float in conjunction with the tide currents and tide stage. It was indicated that the float was dry for approximately one hour longer than would be expected in commercial operations. Dr. Quayle mentioned that the experimental float may be an atypical situation. It was noted that in the second phase commercial floats would also be used.

Messrs Girard and McMillin inquired about bottom samples taken during the float study. Mr. Vasconcelos indicated that in the first experiment the samples at low tide were taken from the bottom and that the values were not significantly different.

A discussion was held between Messrs Bower, Glude, and Beck and Drs. Dollar and Hoff on the significance of the low bacterial counts when the oysters were out of the water.

REFERENCES

American Public Health Association, 1962. Recommended procedures for the bacteriological examination of seawater and shellfish (3rd ed.)

Kelley, P. S., 1964. Brief hydrographic survey of Burley Lagoon, Washington, 1964 Proceedings Northwest Shellfish Sanitation Research Planning Conference. PHS Pub. No. 999-FP-4.

MacLeod, R. A., E. Onofrey, and M. E. Norris, 1954. Nutrition and metabolism of marine bacteria. 1. Survey of nutritional requirements. Presented at 38th Annual Meeting of the Federation of American Societies for Experimental Biology, Atlantic City, New Jersey, April, 1954.

Zerbe, W. B., and C. B. Taylor. 1953. Sea water temperature and density reduction tables. U. S. Dept. of Comm. No. 298.

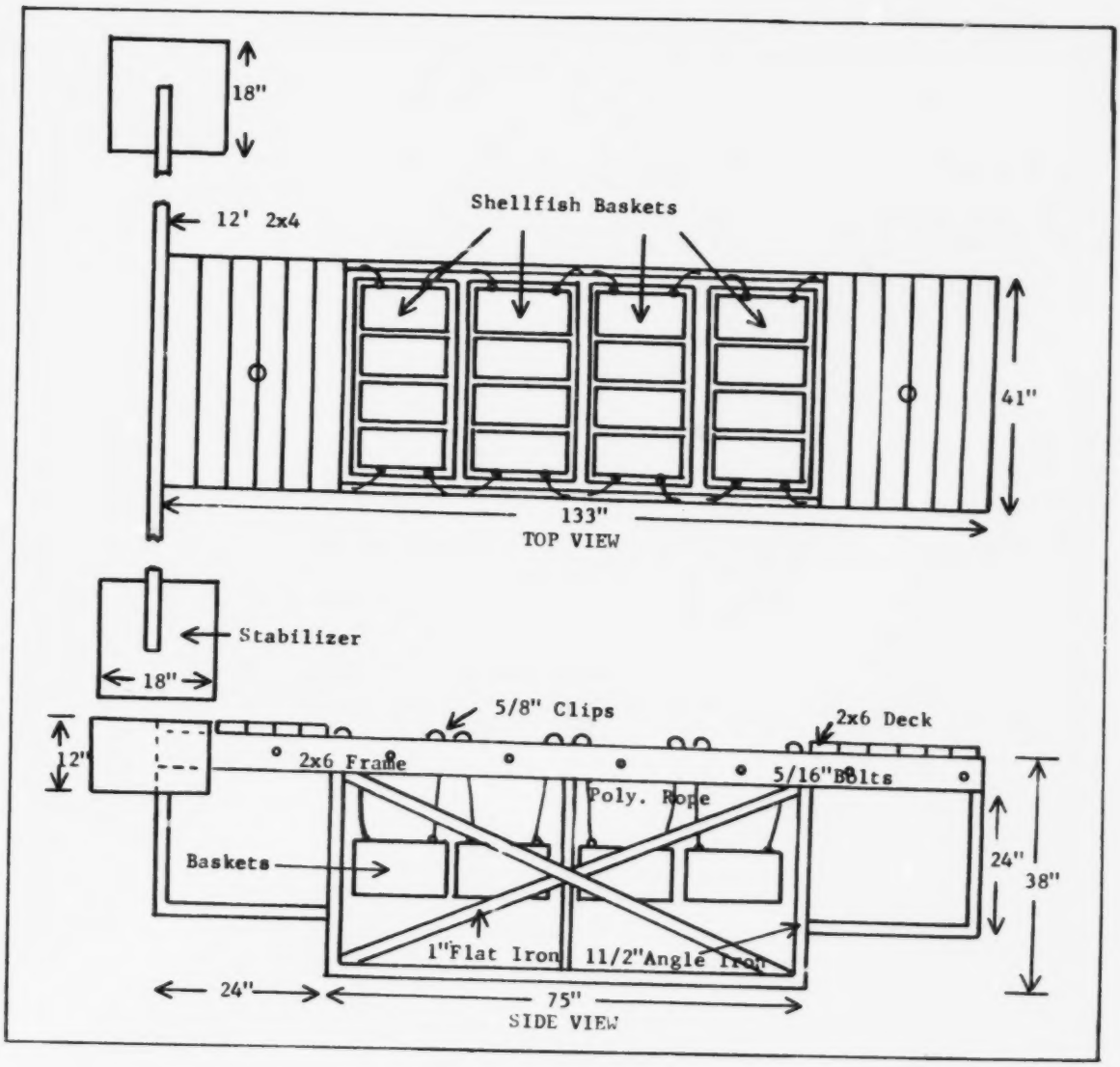

Fig. 1 Shellfish float

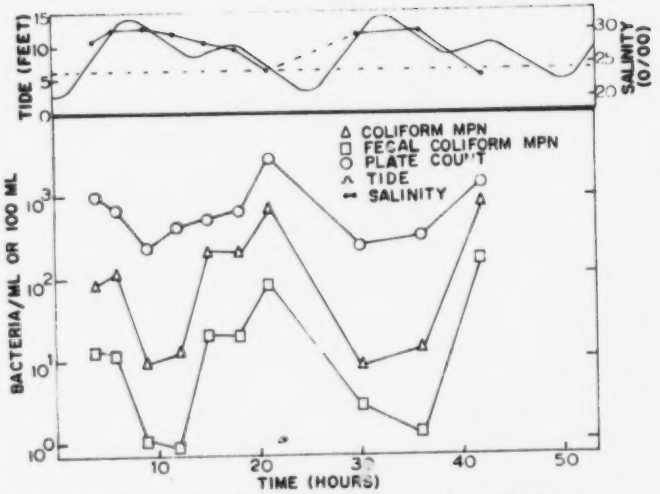

Fig. 3 Bacteriological changes in water
(Run No. 1)

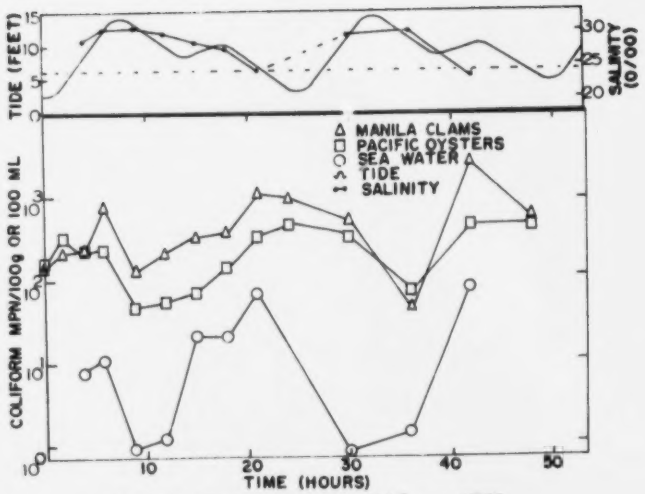

Fig. 4 Changes in coliform MPN
(Run No. 1)

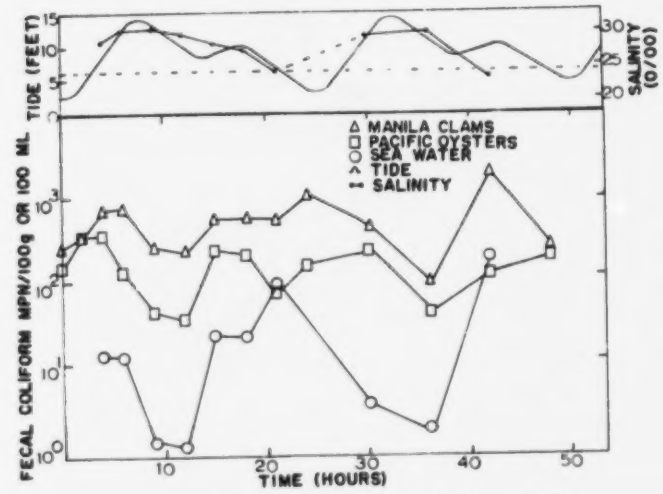

Fig. 5 Changes in fecal coliform MPN
(Run No. 1)

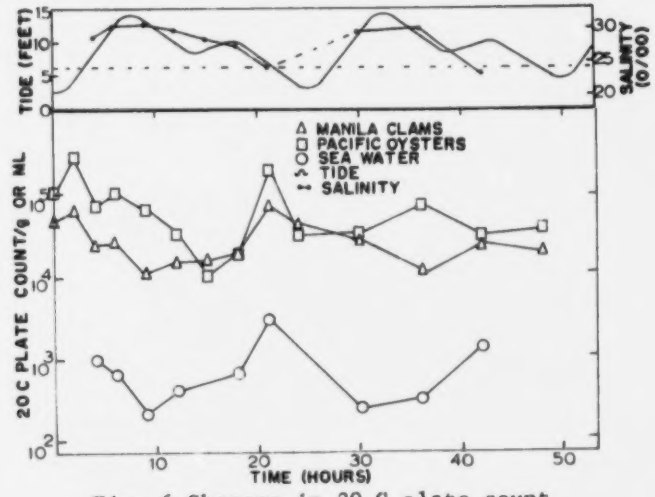

Fig. 6 Changes in 20 C plate count
(Run No. 1)

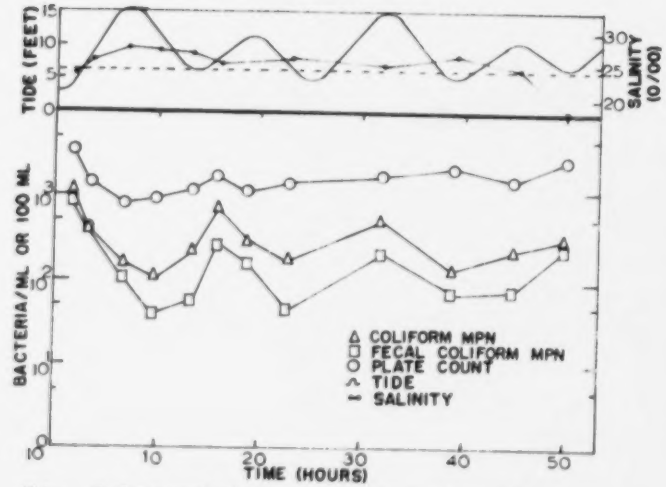

Fig. 7 Bacteriological changes in water
(Run No. 2)

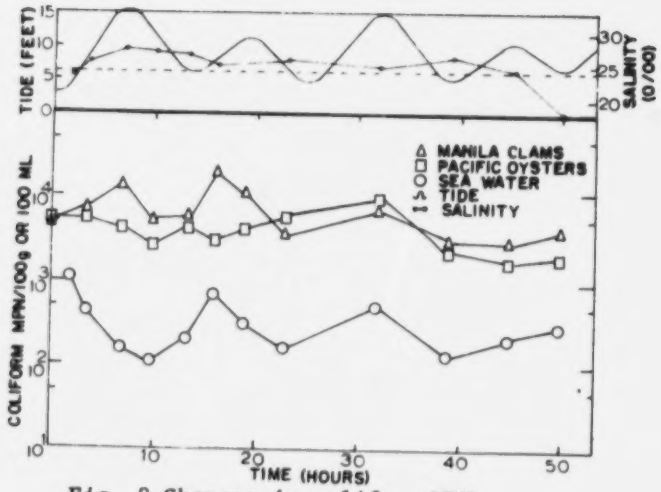

Fig. 8 Changes in coliform MPN
(Run No. 2)

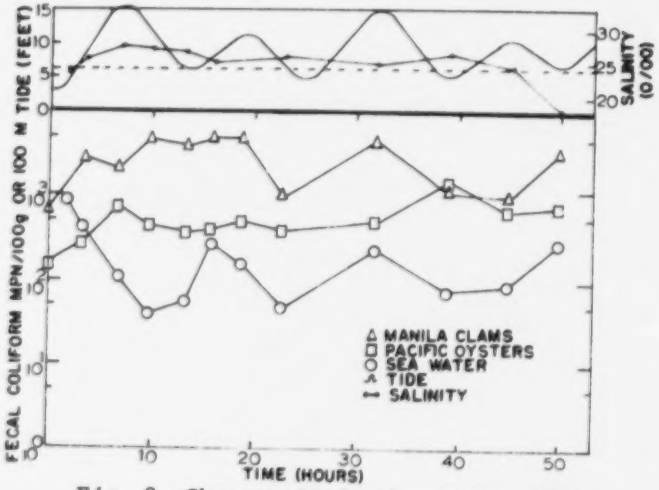

Fig. 9 Changes in fecal coliform MPN
(Run No. 2)

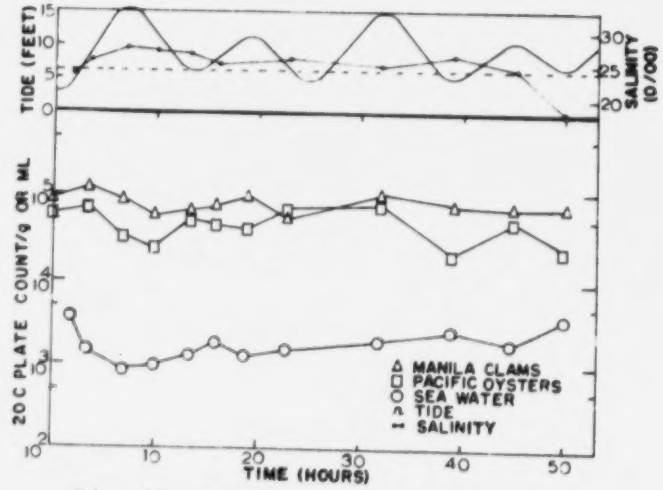

Fig. 10 Changes in 20 C plate count
(Run No. 2)

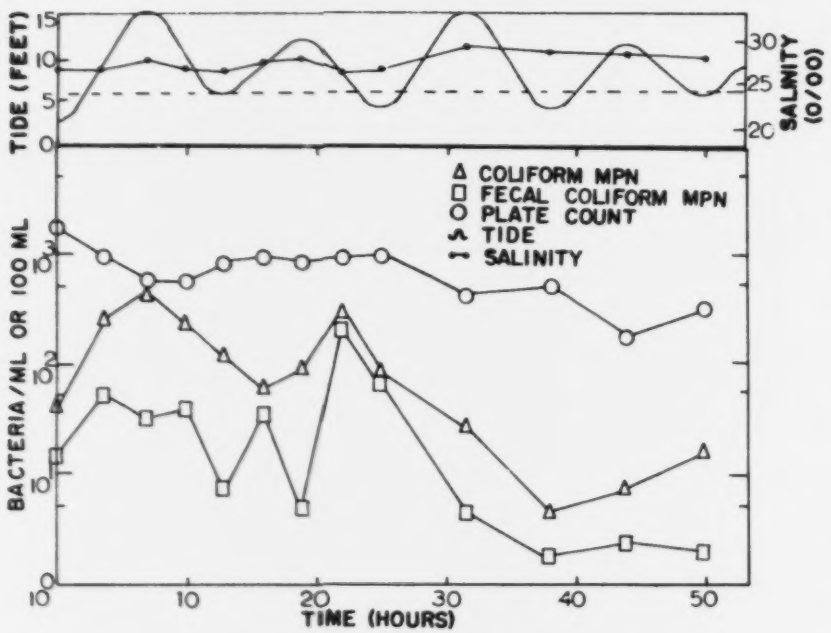

Fig. 11 Bacteriological changes in water
(Run No. 3)

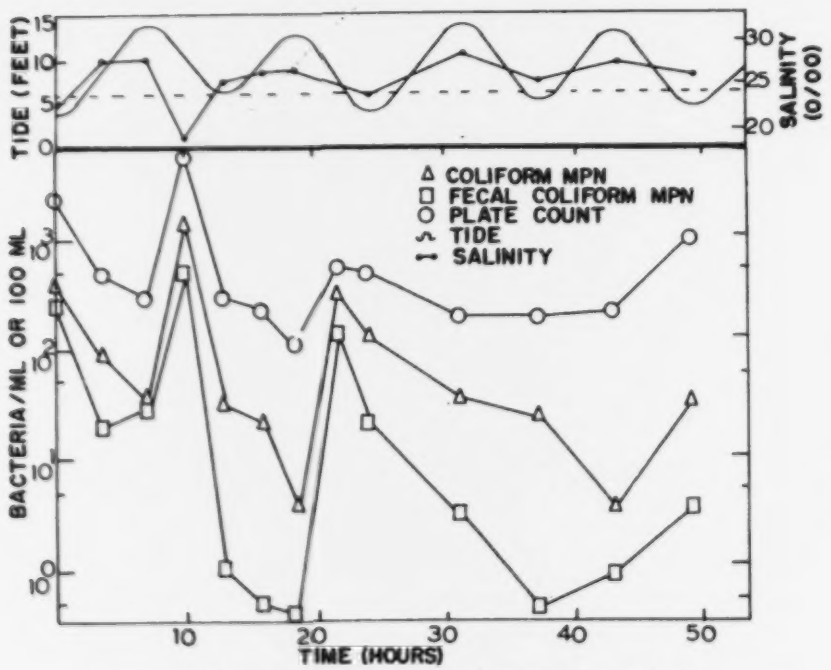

Fig. 12 Bacteriological changes in water
(Run No. 4)

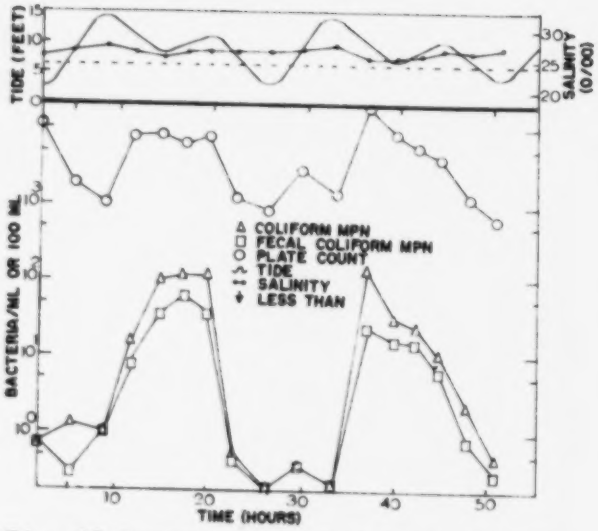

Fig. 13 Bacteriological changes in water
(Run No. 5)

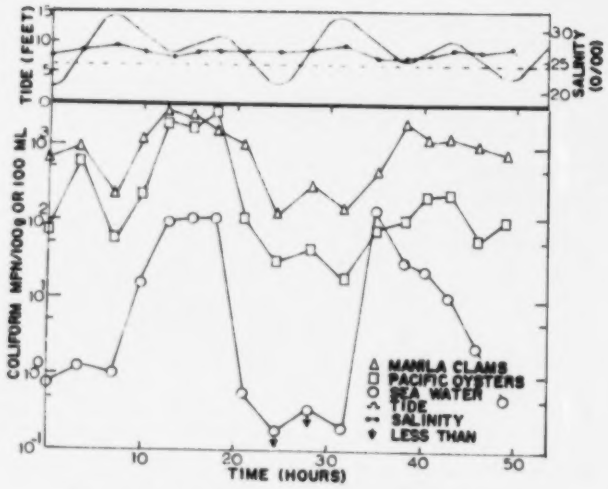

Fig. 14 Changes in coliform MPN
(Run No. 5)

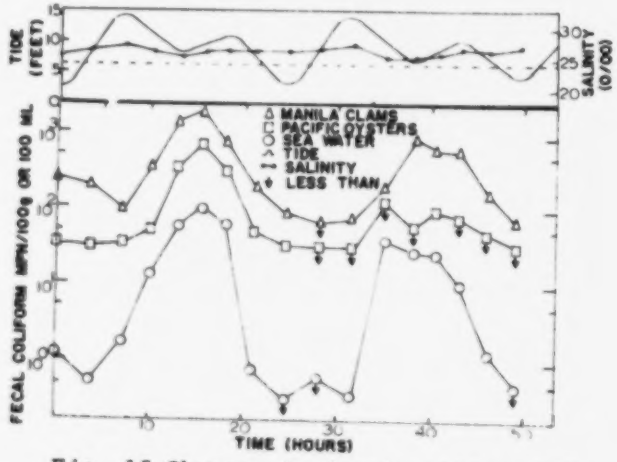

Fig. 15 Changes in fecal coliform MPN
(Run No. 5)

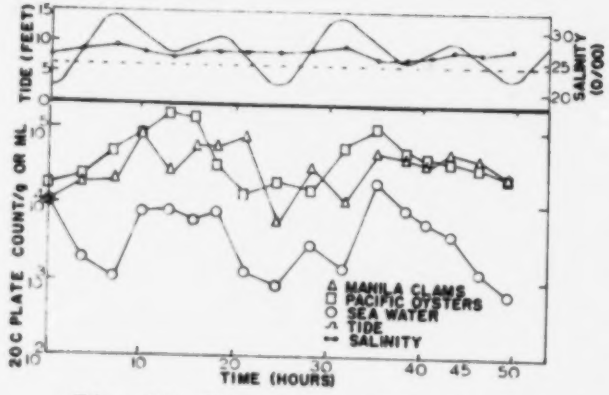

Fig. 16 Changes in 20 C plate count
(Run No. 5)

Table 1. Comparison of float run temperature, salinity and rainfall data

Run No.	Data	Temperature Range[a] (°C)	Salinity Range (o/oo)	Total Rainfall (Inches)
1	Nov.-Dec., 1965	8.7-10.6	23.5-30.8	0.28
2	Jan., 1966	7.3-7.9	18.2-27.6	3.80
3	Feb., 1966	7.0-8.0	26.5-29.3	0.44
4	Mar., 1966	4.0-9.0	18.7-28.5	1.00
5	Jul., 1966	15.0-23.0	25.7-27.2	0.00

[a]Of seawater at float site.

ACCUMULATION OF BACTERIA BY TWO SPECIES OF WEST COAST SHELLFISH MAINTAINED IN AN ESTUARINE ENVIRONMENT

W. Jakubowski

INTRODUCTION

Several investigations on the accumulation of coliform bacteria by shellfish under controlled laboratory conditions have been made. Species used in these experiments have included the Eastern oyster (Crassostrea virginica) (Kelly, et al, 1960), the Pacific oyster (Crassostrea gigas) and Olympia oyster (Ostrea lurida) (Kelly, 1961) and Native Littleneck clams (Protothaca staminea) and Manila clams (Tapes philippinarum) (Beck, et al, 1966). In addition, some observations on accumulation of coliform organisms by the Eastern oyster under natural conditions were made by Presnell and Kelly, (1961).

Studies on wet storage of oyster and clam shellstock are being conducted at this laboratory. These studies are providing information on the immediate or short term response of shellfish to changes in bacteriological and physical conditions during the individual experiments (Jakubowski and Vasconcelos, 1966). Comparison of the overall results of the experiments conducted under various hydrographic and climatological conditions provided additional information. The latter type of comparison is the subject of this paper.

MATERIALS AND METHODS

Experimental Design:

The apparatus, shellfish sampling procedures and methods of examination used in the wet storage studies were described in the preceding presentation (Jakubowski and Vasconcelos, 1966).

Data Analysis:

The data presented in this report are based on geometric means calculated from all results for each index in each experiment. The number of sampling intervals during each experiment were as follows: Experiment one 14, Experiment two 12, Experiment three 13, Experiment four 13, and Experiment five 17. Since duplicate samples were taken at each interval the number of samples represented is twice the number shown above for each experiment.

The values calculated represented the mean level or concentration of each index organism in the water and the two shellfish species during the 48 hr duration of each experiment. Accumulation ratios for each index were also calculated by dividing the mean level in the shellfish by the mean water concentration.

RESULTS

The mean coliform MPN levels of the water, clams, and oysters in each of the five experiments are shown in Fig. 1. Variations in mean coliform levels in the water were fairly large. The difference between levels in experiments 2 and 5 was nearly 100 fold. The mean coliform levels accumulated by the shellfish during the first four experiments were very similar although the mean levels in the water varied considerably. These results also show that mean coliform levels in the clams were higher than those in the oysters in all five experiments.

Mean fecal coliform levels are shown in Fig. 2. The fecal coliform MPN's of the water followed the same general pattern as was shown by the coliform MPN's and the difference in fecal coliform level between experiments 2 and 5 was of the same magnitude as the coliform difference. In this case also mean levels accumulated by the shellfish were more uniform than the levels in the water. Fecal coliform levels in the clams were consistently higher than in the oysters.

The results using mean 20 C plate count levels are shown in Fig. 3. Levels of this index in the water were much more uniform than the coliform and fecal coliform levels. In addition, the highest plate count levels were shown in experiment 5 when coliform and fecal coliform levels were lowest. The plate count levels in both shellfish species were also much more uniform than were the coliform and fecal coliform levels. In all except one experiment, oysters showed higher levels of organisms enumerated by the 20 C plate count than did clams.

The accumulation ratios of the means shown by the two species are shown in Table 1. The lowest coliform and fecal coliform accumulation ratios in both species occurred in experiment 2. During this experiment, water temperatures were lower than in any of the other four experiments. Nearly 4 inches of rain with a corresponding large variation in salinity also occurred during this experiment. However, in experiment 4, during which water temperatures attained only slightly higher levels and a similar wide range in salinity occurred, much higher mean coliform and fecal coliform ratios occurred.

When coliform and fecal coliform ratios are ranked in increasing order they follow similar patterns in both species with the exception of experiment 5. In this experiment accumulation ratios of the clams were very high while those of the oysters were much lower. It is also interesting to note that the clams showed the lowest coliform and fecal coliform ratios when the levels of these indices in the water were highest (Exp. 2) and the highest ratios when the levels of these groups in the water were the lowest (Exp. 5).

An entirely different pattern is shown by the 20 C plate count ratios. The accumulation ratios are more uniform throughout the series of experiments. However, the highest ratios again occurred in both species when 20 C plate count levels in the water were lowest (Exp. 4) and the lowest ratios occurred when levels in the water were highest (Exp. 5).

DISCUSSION

Coliform and fecal coliform levels in the water followed a pattern to be expected in the Pacific Northwest. Levels were much higher during the winter and spring months when considerable runoff occurred and much lower in midsummer when little precipitation and consequent runoff occurred. In contrast the 20 C plate count levels in the water remained fairly constant. Although the level was constant the composition of the species making up the flora undoubtedly varied with changing temperatures, nutrients available, and other factors.

As indicated in the previous paper (Jakubowski and Vasconcelos, 1966) changes in bacterial concentration in both water and shellfish occurred rapidly during each experiment. In view of the lag and lesser degree of change shown by the shellfish the validity of the use of mean ratios derived from all the results in each experiment may be questioned. However, the sampling intervals and the number of tide cycles were similar in each experiment. Also, the lag in shellfish response occurred during both increases and decreases in bacteriological levels in the water. Therefore, the low and high ratios observed at these extremes would tend to offset each other.

The accumulation ratios shown in these experiments were considerably higher than those observed previously in laboratory experiments using these same species. In a series of 11 such experiments conducted by Kelly (1961) the highest average accumulation ratio obtained with Pacific oysters was less than 10 fold. In another series of 12 experiments, the highest average ratio attained by Manila clams was 38 fold (Beck et al, 1965). Under laboratory conditions, Eastern oysters showed accumulation ratios as high as 16 fold (Kelly, Arcisz, and Presnell, 1960). Later studies by Presnell and Kelly (1961) indicated that Eastern oysters sampled under natural conditions showed accumulation ratios similar to those attained under laboratory conditions.

Several factors may have contributed to the high accumulation ratios observed in these experiments. As indicated above, bacterial levels in the shellfish usually did not decline as rapidly as the levels in the water which would tend to make the shellfish mean levels higher than those of the water. Holding the shellfish suspended in the water rather than on the bottom may have had some effect but during two of the experiments, oyster samples were taken from the bed at low tide in the vicinity of the float. Since these oysters showed bacterial levels similar to those of oysters in the float sampled at the same time it appears that this may not have been a factor. Concentration of microorganisms present in the water during these experiments may have been a factor. Mean coliform MPN's ranged from 4.5 to 310 and fecal coliform MPN's from 2.5 to 150, while the laboratory experiments referred to above were usually conducted at MPN levels of about 1,000. The inverse relationship between bacterial levels in the water and levels in the oysters was pointed out above. However, it is difficult to perceive how differences in bacterial concentration in this range could influence accumulation.

It should be pointed out that this investigation is still in progress. The five experiments conducted to date have revealed some significant information relative to accumulation and elimination of bacteria by shellfish. However, more experiments and further analysis of the data accumulated will be necessary to identify the causes of the variations observed.

COMMENTS BY PARTICIPANTS

Mr. McMillin inquired about the relationship of the bacterial content of shellfish to that of the overlying water. Dr. Sparks indicated that the rates of uptake should be lower in the winter than in the summer due to slower physiological activity of the shellfish. However, the bacterial values of the overlying water were much higher in the winter and this may counteract the reduced physiological activity.

Miss Campbell asked if 35 C plate counts were included. Dr. Hoff replied that only 20 C plate counts were made. The plate counts were more stable throughout the study than the MPN's for coliform or fecal coliform organisms.

REFERENCES

Beck, W. J., C. B. Kelly, J. C. Hoff, and M. W. Presnell. 1966. Bacterial depuration of West Coast shellfish. Proc. First National Depuration Conf. In press.

Kelly, C. B., W. Arcisz, M. W. Presnell, and E. K. Harris. 1960. Bacterial accumulation by the oyster, Crassostrea virginica, on the Gulf Coast. R. A. Taft San. Eng. Center Tech. Rept. F60-4.

Kelly, C. B. 1961. Accumulation of bacteria by the Pacific and Olympia oysters. (Paper presented at Shellfish Sanit. Res. Conf., Purdy, Wash.)

Presnell, M. W. and C. B. Kelly, 1961. Bacteriological studies of commercial shellfish operations on the Gulf Coast. R. A. Taft San. Eng. Center Tech. Rept. F61-9.

Jakubowski, W., and G. J. Vasconcelos. 1966. Studies on wet storage of oyster and clam shellstock. (See p.41, These Proceedings)

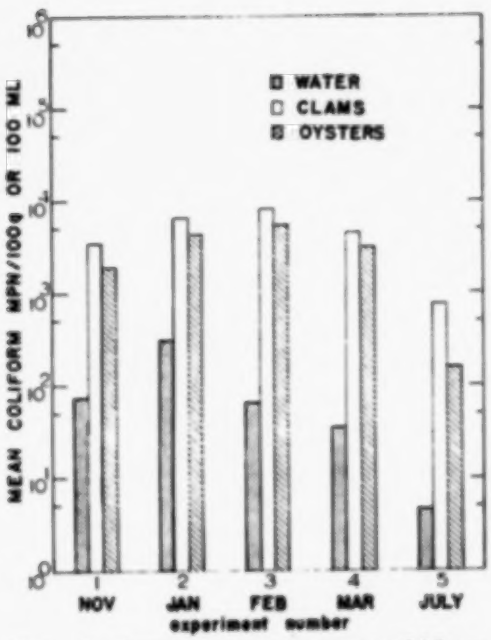

Fig. 1 Comparison of mean coliform MPN's of shellfish and seawater from five float runs

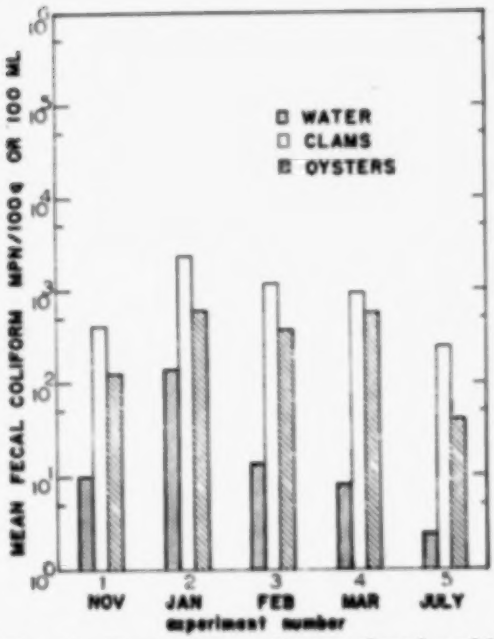

Fig. 2 Comparison of mean fecal coliform MPN's of shellfish and seawater from five float runs

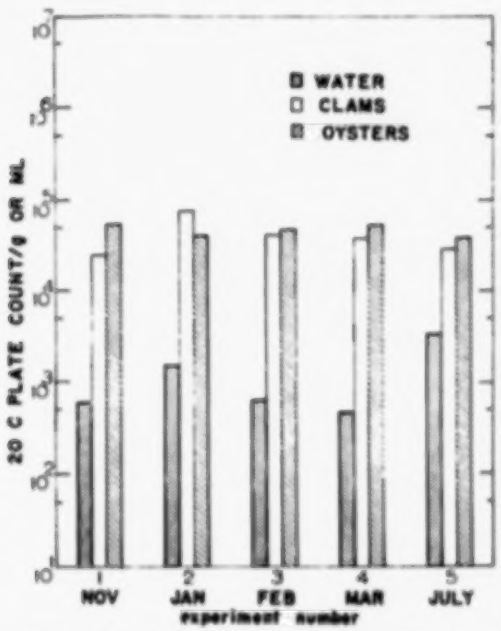

Fig. 3 Comparison of mean 20 C plate counts of shellfish and seawater from five float runs

Table 1. Coliform, fecal coliform and 20 C plate count accumulation ratios of Pacific oysters and Manila clams

Experiment No.	Temp. Range (C)	Salinity Range o/oo	Total Rainfall (inches)	Accumulation Ratio: Concentration in shellfish / Concentration in water					
				Coliform MPN		Fecal Coliform MPN		20 C Plate Count	
				clams	oysters	clams	oysters	clams	oysters
1	8.7-10.6	23.5-30.8	0.28	50	26	41	13	43	90
2	7.3- 7.9	18.2-27.6	3.80	22	14	16	4	45	24
3	7.0- 8.0	26.5-29.3	0.44	120	85	86	27	63	72
4	4.0- 9.0	18.7-28.5	1.00	130	83	120	72	78	100
5	15.0-23.0	25.7-27.2	0.00	160	38	100	16	9	12

CHEMICAL AND BIOLOGICAL ANALYSIS OF SHELLFISH AND SEAWATER AT SITE OF NORTHWEST SHELLFISH SANITATION RESEARCH CENTER

W. J. Beck

INTRODUCTION

The principal purpose of the Northwest Shellfish Sanitation Research Center will be to conduct basic research, technological studies and technical consultation, and assistance associated with the National Shellfish Sanitation Program. Broad general areas of investigation will include:

a) the fate of bacteria and viruses in marine estuaries.

b) the accumulation, retention and elimination of bacteria and viruses in shellfish.

c) the biological and biochemical fundamentals of depuration.

d) the role of chemical and radiobiological pollutants in marine estuaries.

e) the physiology and ecology of shellfish poisons.

f) the significance of pesticides, toxic or organic chemicals and metals.

g) the public health significance of human parasites in shellfish.

In order to conduct these various phases of research, the prime criteria for the location of the site of the NWSSRC were set as follows:

> "The site must be located on the shore of a marine area capable of supporting the growth of commercially important marine species of bivalve molluscs. The seawater supply must be of excellent sanitary and biological quality and there must be reasonable assurance of the continued good quality of this water in the foreseeable future. The supply must be free from industrial, domestic or other pollution which would impart to the water a biological quality adverse to the normal behavior of shellfish."

Through the cooperation of the Washington State Department of Health and Commission of Parks and Recreation, the Manchester site came under consideration in February 1965. In order to determine whether or not this site would meet the above criteria, bacteriological, physical, chemical and biological tests were initiated. The study of several of these

parameters was made possible through the cooperation of the Washington State Departments of Health and Fisheries, University of Washington College of Fisheries and School of Oceanography and the PHS Northeast Shellfish Sanitation Research Center.

METHODS AND MATERIALS

Seawater Sampling:

A sampling station was located at the northeast end of the pier shown in Fig. 1. Water samples were taken at three depths. These were one foot below the surface, 18-22 feet below the surface and 35-40 feet below the surface. The total depth at mean high tide was approximately 50 feet. Water samples were collected in sterile ground glass stoppered bottles. The sampling device was that described by Kelly (1958).

Bacteriology:

All samples for bacteriological examination were immediately transported to the laboratory. Water samples were examined for coliform and fecal coliform organisms by the 5 tube multitube method. Oyster samples were examined for coliform and fecal coliform organisms by the 5 tube multitube method. In addition, 35 C plate counts on standard methods agar were conducted. All bacteriological methods were those described by APHA Recommended Procedures for the Bacteriological Examination of Seawater and Shellfish, 3rd edition (1962).

Salinity and Temperature:

Salinity and temperature were measured immediately after taking the bacteriological samples. The instrument used for these observations was a Model RS5-2 Electrodeless Induction Salinometer, serial number 124, manufactured by Industrial Instruments, Inc. of Cedar Grove, New Jersey. The instrument has been described by Kelley (1965).

Bioassays:

Seawater samples were collected off the end of the pier at 1 and 30 foot depths for Bioassays by the Oyster Larvae Technic (Woelke, 1960). The water samples were packed in ice and transported to the Shellfish Laboratory, Washington State Department of Fisheries, Brinnon, Washington, for analysis. Samples were collected in April and June 1965.

Chemical Analysis:

Pacific oysters, Crassostrea gigas, were collected from commercial beds in Burley Lagoon, Washington. From a total of 400 individual animals, approximately 200 were placed in a flowing seawater aquarium at

the Purdy laboratory. The remaining 200 oysters were suspended in a basket at a depth of approximately 20 feet from the bottom off the end of the pier at the Manchester site. Oyster samples were taken at 4 week intervals for a total of 20 weeks. Sister samples from the Purdy tank were collected at the same intervals. The shellstock was frozen and forwarded to the Northeast Shellfish Sanitation Research Center. The oysters were analyzed for the following metals: zinc, copper, manganese, iron, lead, cobalt, nickel and chromium (Pringle, 1965). To date, the study has continued from March 1965 through June 1966.

Oceanography:

Tide patterns and currents have been determined by the use of drift bottles in the immediate vicinity of the pier at the Manchester site. Personnel were stationed in a small boat and observations were taken through several complete tide cycles to determine the current patterns in the vicinity of the pier. In addition, one cruise aboard the University of Washington research vessel "Commando" has been made. On this cruise, preliminary soundings were made to determine the contour of the bottom.

RESULTS

Bacteriological Analysis:

Probability plots were made on a yearly basis for the coliform and fecal coliform MPN's of the seawater samples at the three depths. The first series of samples was taken between February and December 1965; the second series between January and August 1966. The results for various coliform percentiles are shown in Table 1 and fecal coliform in Table 2.

The range of coliform MPN's for ebb tide samples at the top and middle depth samples was similar throughout the entire study. The greatest range was from a coliform MPN of .58/100 ml at the 10 percentile level to 55/100 ml at the 90 percentile level. The bottom depth coliform MPN range was somewhat different in 1965 and 1966. In 1965 the coliform MPN range was from 1.6 to 30/100 ml, whereas in 1966 the range was greater (.26 to 62/100 ml). The 50 percentile level for all ebb tide samples was 6.8/100 ml at the top and bottom and 9.2/100 ml for the middle samples.

The flood tide sample ranges were quite similar to those observed for the ebb tide. The 50 percentile level for all flood tide samples was 2.6/100 ml at the top; 6.0/100 ml for the middle and 3.7/100 ml for the bottom. However, the only coliform MPN's above 230/100 ml were found during the flood tide. Of the ten samples that were above

230/100 ml, 7 were collected between February 23, 1965 and March 11, 1965. The other three high counts were obtained on one sample collected on January 17, 1966. Eight of the 10 high samples were found in the top and middle depth locations.

Fecal coliform MPN ranges were considerably lower than those found for the coliform MPN's. The greatest range was for the top and middle depth flood tide samples in 1965 and the ebb tide samples of 1966. The 50 percentile level for fecal coliform MPN's for all samples was from .70/100 ml to 1.7/100 ml.

During 1966, a total of eight samples of Pacific oysters, Crassostrea gigas, was analyzed for coliform and fecal coliform organisms plus 35 C plate counts. The geometric mean for the coliform MPN's was 1100/100g and the 35 C plate count was 600/g. As indeterminately low values were found for the fecal coliform MPN the median was determined at 170/100g.

Bioassay of Seawater:

The results of two bioassays of the seawater from the NWSSRC as analyzed by the Shellfish Laboratory, Washington State Department of Fisheries, Brinnon, Washington, are shown in Table 3. The assay performed in April 1965 showed an exceedingly low number of abnormal oyster larvae. The assays in June 1965 were somewhat higher. The water sample from the top had 7.44% and from the 30 foot depth had 2.95%. According to Woelke (1960) these later results were still within the limits set for the assay method.

Chemical Analysis:

The results of chemical analysis for trace metals on Pacific oysters, Crassostrea gigas, held at Manchester,and the Purdy laboratory, are shown in Table 4. Oysters were examined on a continuing basis. The first lot was from March-June 1965, the second from July to December 1965 and the third lot was from December 1965 to June 1966. All values for lead, cobalt, nickel and chromium were below those measurable by the technics used. The range of zinc was constant for the first and third phase (155-220mg/kg wet weight and 160-260mg/kg wet weight respectively) and slightly higher during the second phase (175-290mg/kg wet weight). Copper and manganese gave similar differences by phase to that found for zinc. The values for iron remained quite constant throughout the study. The lowest value for iron was 16mg/kg wet weight and the highest 38mg/kg wet weight. No appreciable difference has been shown between the oysters stored at Manchester with those held in the aquarium at the Purdy laboratory.

Physical Analysis:

The range of temperature and salinity variances at middle depth from January 1965 through August 1966 are shown in Fig. 2.

The temperature range was found to be lowest during January-February (7.0 C). The temperature of the seawater remained at 8 C or less through March. During the months of April, May and June there was a gradual warming period until a high of 12.5-13.5 C was recorded during July and August. This was followed by a gradual lowering of the temperature through the month of December to 10 C. The range of temperatures recorded through the two-year study was from 7.6-13.8 C.

Recordings of salinities ranged from 28 to 30 o/oo during the January to July period. One low salinity reading of 26.9 at the top during a flood tide was recorded in February 1965. In the latter part of August through December the salinity at middle depth rose to a range from 30 o/oo to 31.5 o/oo. The salinity pattern did not present the same changes as recorded by the temperature of the salt water.

Oceanography:

Current directions were made by means of drift floats at 6 ft., 18 ft., and 30 ft. respectively. The general pattern of ebb and flood tides was found to be similar. Patterns shown through the ebb tide cycle are shown in Figs. 3 and 4.

During the first half of the ebb tide, the currents within 100 yards of the pier at Clam Bay were predominately in a counterclockwise direction. The major passage of water was from Rich Passage into middle Puget Sound proper. At about mid ebb tide the currents reversed to a clockwise direction as shown in Fig. 4. At the same time the major flow of water continued from Rich Passage into middle Puget Sound. The same change in pattern was noted on the flood tide. Thus, in one complete tidal cycle two alternating current patterns were observed. Mixing with waters, traveling to and from Rich Passage would be almost continuous.

DISCUSSION

In order to determine if the site for the Northwest Shellfish Sanitation Research Center would meet certain criteria, various bacteriological, biological, chemical, physical and oceanographic data have been collected since early 1965. To date, the results of this data collection have shown that the site meets the criteria for the research center. Although minor variation has been shown in

bacteriological and biological data, the variance is not enough to preclude the use of the seawater for proposed research purposes. Chemical and physical properties tested to date are well within the tolerances set for individual parameters.

All of the above studies will be continued. In addition, through the cooperation of the University of Washington and the U. S. Department of Commerce, the oceanography studies will be expanded. One preliminary sounding tour of the estuarine bottom has been completed. Further utilization of the research vessel "Commando" will be made to verify these soundings as well as determine the composition of the bottom, plus temperature and salinity variance in the area. Several monitoring buoys are to be located in the area by the Department of Commerce. Thus a continuous recording of salinity, temperature and current will be made at approximately six locations. A continuous recording salinometer will also be placed at mid depth off the pier of the NWSSRC site. Three thermographs are now in continuous use at the same site.

With the continuous monitoring of all these parameters, valuable background information will be available to be included in many of the research projects of the Northwest Shellfish Sanitation Research Center.

COMMENTS BY PARTICIPANTS

Mr. Glude asked about stratification of water temperatures at the Manchester site. Mr. Beck replied that there was little difference noted at the three depths tested. Dr. Dollar inquired about oyster larvae bioassays and evidence of organic matter coming from south of the site. Mr. Beck stated that only two oyster larvae bioassays had been made to date and others are scheduled in the near future. The only variation in parameters tested to date occurred in January and February of 1965 and 1966.

At this time Mr. Felsing reported on his Alaska trip in relationship to the establishment of a field station to study marine toxins. Dr. Quayle indicated that Canada was glad to hear of the interest in such a station in Alaska and that it could supplement the work done in Canada.

REFERENCES

American Public Health Association. 1962. Recommended procedures for the bacteriological examination of seawater and shellfish, 3rd edition.

Kelley, P. S. 1965. Brief hydrographic survey of Burley Lagoon, Washington. Proc. N. W. Shell. Sanit. Res. Plann. Confer. PHS Pub. No. 999-FP-4.

Kelly, C. B. 1958. Bacteriological examination of shellfish and seawater. Shellfish Sanitation Course Manual, Robt. A. Taft San. Engr. Center, U. S. Dept. Health, Education, and Welfare, Public Health Service, Cinn. Ohio.

Pringle, B. H. 1965. Unpublished data, Northeast Shellfish Sanitation Research Center, Narragansett, R. I.

Woelke, Charles E. 1960. Effects of sulfite waste liquor on the normal development of the Pacific oyster (Crassostrea gigas) larvae. Wash. Dept. of Fish. Res. Bull. No. 6. pp 149-161.

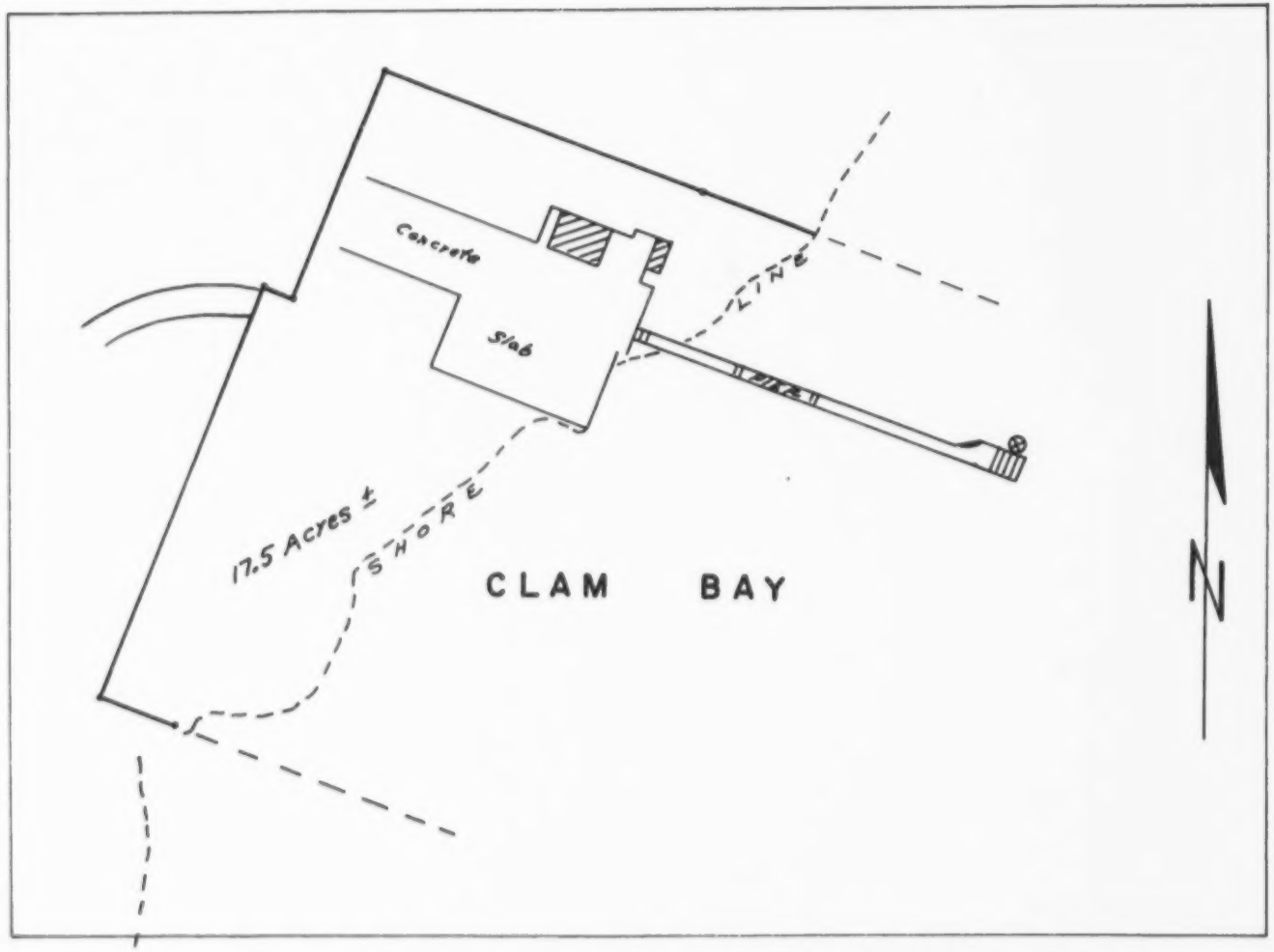

FIG. 1 PROPOSED SHELLFISH SANITATION RESEARCH CENTER
(MANCHESTER, WASHINGTON)

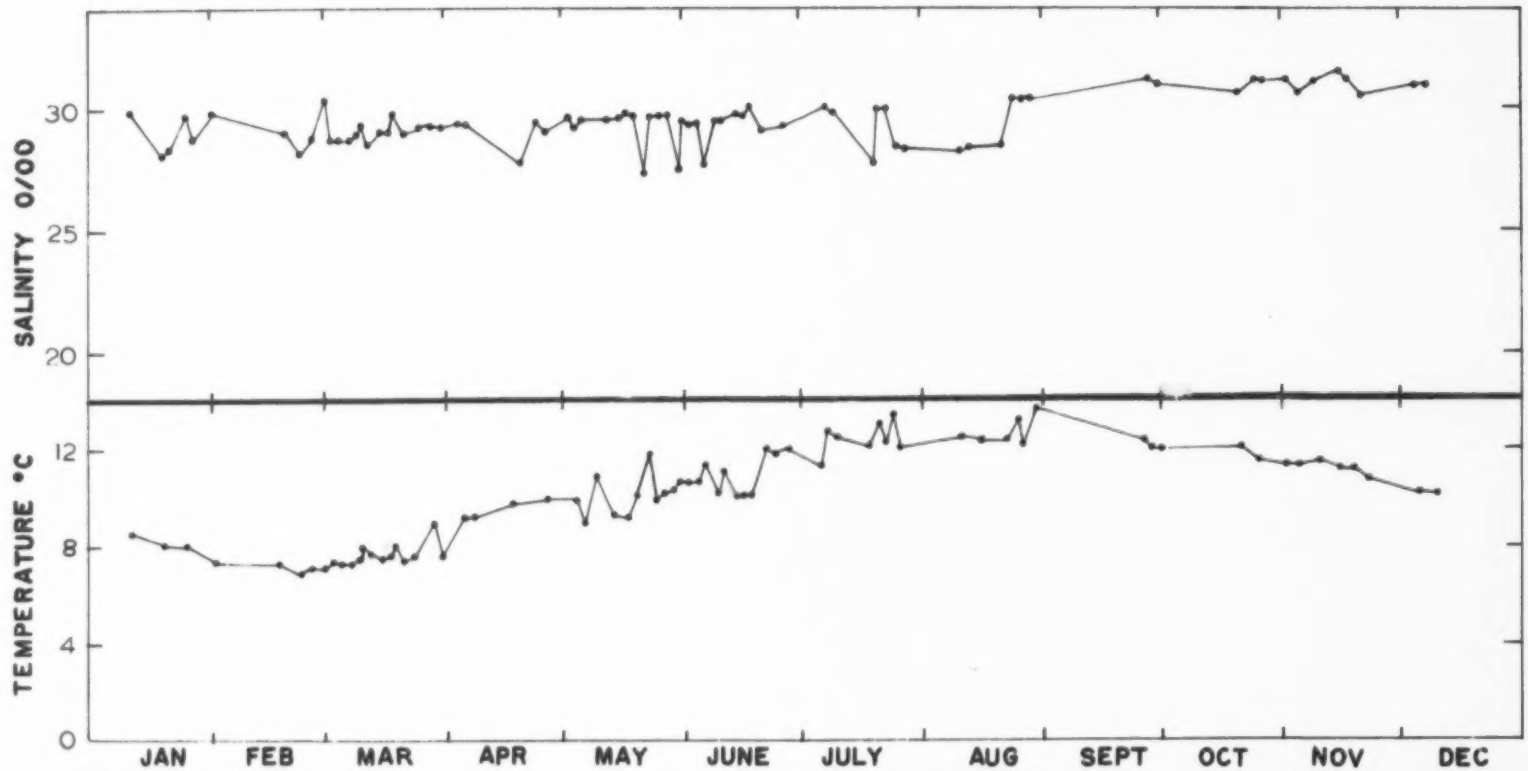

FIG. 2 SALINITY AND TEMPERATURE RECORDINGS AT NWSSRC FROM JAN 1965 THROUGH AUG 1966

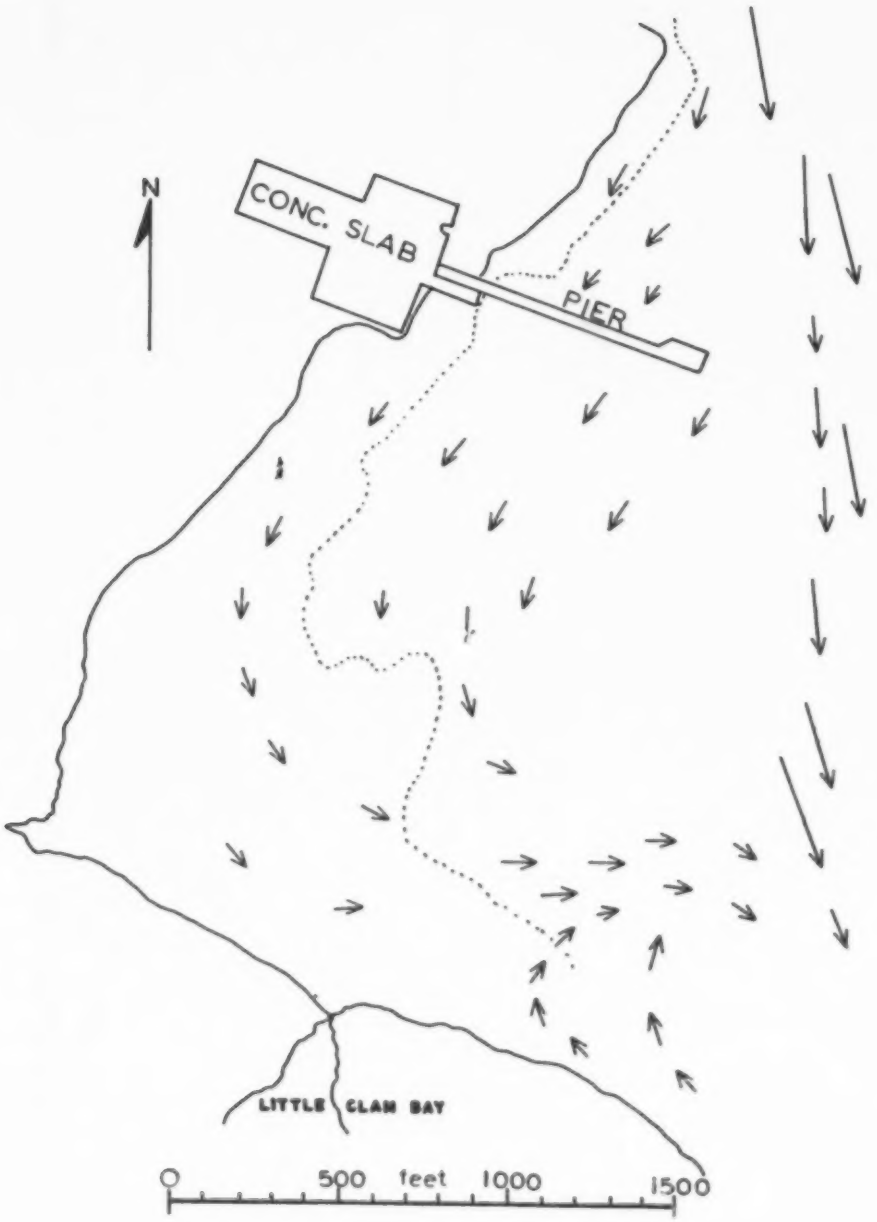

FIG. 3 CURRENT PATTERNS DURING FIRST HALF OF EBB TIDE (+10ft TO +4ft)

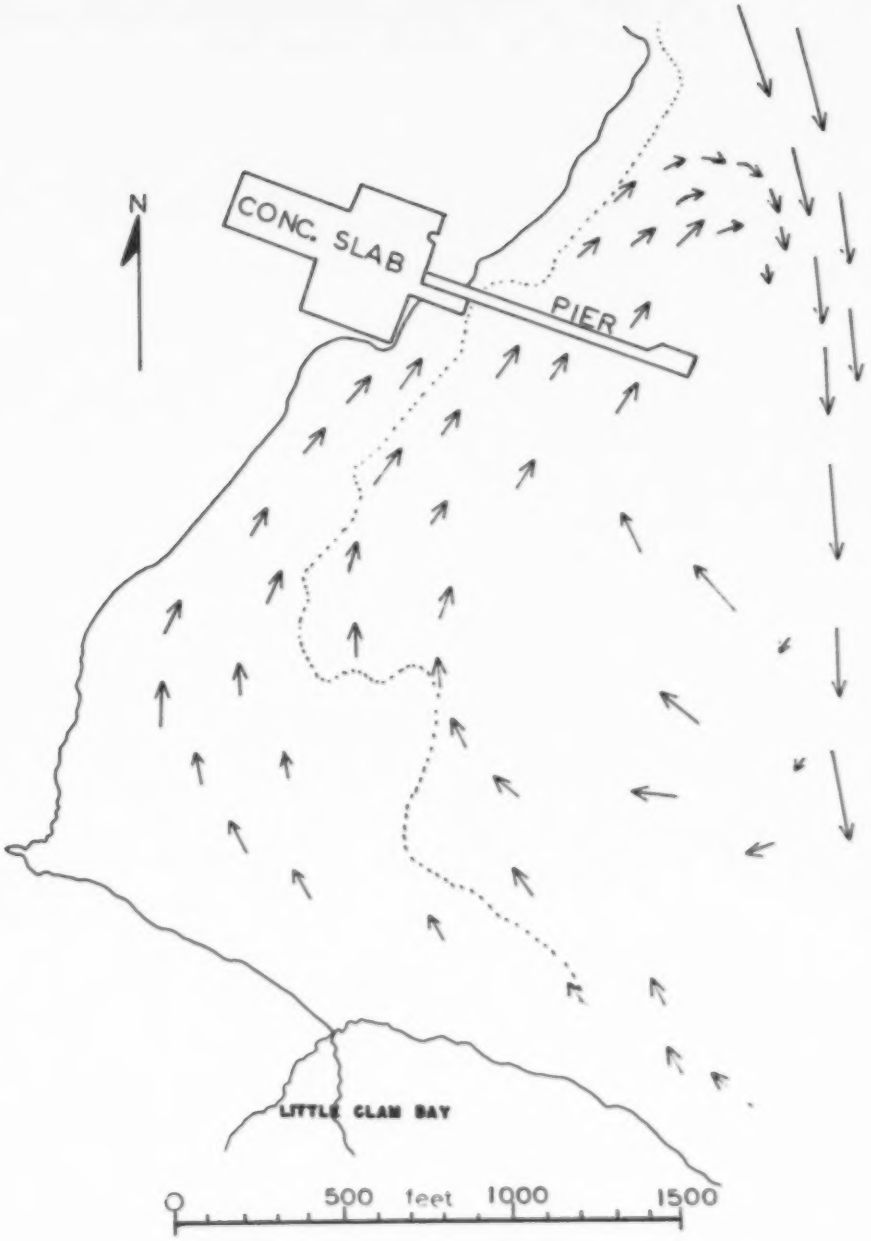

FIG. 4 CURRENT PATTERNS DURING SECOND HALF OF EBB TIDE (+4ft TO -2ft)

Table 1. Coliform MPN's on seawater samples from Northwest Shellfish Sanitation Research Center Site

Date	Tide	Depth	No. of Samples	Probability percentile of coliform MPN's/100 ml seawater			
				10	50	70	90
Feb./Dec. 1965	Ebb	Top	30	.88	6.2	14	45
		Middle	30	1.4	8.4	17	47
		Bottom	28	1.6	7.0	13	30
Jan./Aug. 1966	Ebb	Top	14	1.0	7.1	16	49
		Middle	14	.58	5.6	15	55
		Bottom	14	.26	4.1	12	62
Feb./Dec. 1965	Flood	Top	31	.40	5.4	16	74
		Middle	31	.82	6.4	15	50
		Bottom	31	.32	4.0	11	50
Jan./Aug. 1966	Flood	Top	17	.45	4.5	11	45
		Middle	16	.68	5.1	11	37
		Bottom	16	.58	3.2	6.4	17
Total 1965-66	Ebb	Top	44	1.0	6.8	15	45
		Middle	44	1.6	9.2	19	55
		Bottom	42	1.1	6.8	15	41
Total 1965-66	Flood	Top	48	<.18	2.6	9.0	54
		Middle	47	.48	6.0	17	72
		Bottom	47	.26	3.7	11	54

Table 2. Fecal coliform MPN's on seawater samples from Northwest Shellfish Sanitation Research Center Site

Date	Tide	Depth	No. of Samples	Probability percentile of fecal coliform MPN's/100 ml seawater 10	50	70	90
Feb./Dec. 1965	Ebb	Top	30	.33	2.0	4.0	11
		Middle	30	.32	2.3	5.0	17
		Bottom	28	.30	2.1	4.4	14
Jan./Aug. 1966	Ebb	Top	14	<.18	1.3	4.3	24
		Middle	14	<.18	2.0	5.5	24
		Bottom	14	<.18	.98	2.9	14
Feb./Dec. 1965	Flood	Top	31	<.18	1.3	4.4	27
		Middle	31	<.18	1.1	4.1	27
		Bottom	30	<.18	1.3	3.4	13
Jan./Aug. 1966	Flood	Top	17	<.18	1.3	2.9	9.6
		Middle	16	<.18	.98	2.4	8.6
		Bottom	16	<.18	.74	2.6	16
Total 1965-66	Ebb	Top	44	.20	1.6	3.6	12
		Middle	44	<.18	1.3	4.2	15
		Bottom	42	.18	1.7	3.2	12
Total 1965-66	Flood	Top	48	<.18	1.2	4.5	32
		Middle	47	<.18	.84	3.7	32
		Bottom	46	<.18	.70	2.4	14

Table 3. Results of bioassays of seawater samples*

Location of Sample	Percent abnormal oyster larvae 1st test	2nd test
Control	0.37	0.76
Burley Lagoon	0.36	-
Manchester 1 ft.	0.00	7.44
Manchester 30 ft.	0.19	2.95

*Bioassays performed by Personnel Shellfish Laboratory, Washington State Department of Fisheries, Brinnon, Washington.

Table 4. Range of West Coast metal values on Pacific oysters* (mg/kg wet weight)

Metal	Phase I March-June		Phase II July-December		Phase III December-June	
	Manchester	Purdy	Manchester	Purdy	Manchester	Purdy
Zinc	155-220	165-230	175-290	225-300	160-260	150-245
Copper	16-17	13-18	12-27	15-23	11-26	11-18
Manganese	2-5	2-5	8-16	6-11	4-6	3-6
Iron	25-37	23-38	27-38	23-37	25-37	16-37
Lead	<.2	<.2	<.2	<.2	<.2	<.2
Cobalt	<.2	<.2	<.2	<.2	<.2	<.2
Nickel	<.2	<.2	<.2	<.2	<.2	<.2
Chromium	<.2	<.2	<.2	<.2	<.2	<.2

*Chemical determinations performed by Personnel Northeast Shellfish Sanitation Research Center, Narragansett, Rhode Island.

PART II

PROPOSED RESEARCH ACTIVITIES FOR

FISCAL YEAR 1967

PROPOSED RESEARCH ACTIVITIES

FOR FY 1967

W. J. Beck

INTRODUCTION

In the past this portion of our program has been of great importance to us. Your constructive criticism of our proposed projects has been helpful in directing our research programs. We hope you will continue with your valuable guidance on the proposals presented at this time.

We also plan to publish the proceedings of this Conference as was done last year.

PROPOSAL

Ecological Studies

The ecological study will be continued by the investigation of the environmental factors affecting the incidence of Clostridium botulinum Type E in an estuarine area. The estuarine area will be selected on the basis of previous indication of the presence of C. botulinum Type E. By the examination of a variety of samples such as animal feces, virgin soil, soil under agricultural use, and fresh water and estuarine muds, the origin or foci of this organism in the estuary or its contributing watershed will be investigated. The implication of such a site of origin as a continuous or intermittent source will also be studied. The effect of factors such as land use, temperature, salinity, rainfall and possible seasonal variation on the incidence of this organism will be considered. These data will be obtained by periodic sampling from selected sites in the estuary, contributing watershed and the surrounding land areas. Improvement of methods for the isolation and enumeration of C. botulinum and its spores from various sources will also be investigated.

COMMENTS BY PARTICIPANTS

Mr. Dupuy asked about ability to sample water containing suspended matter. Mr. Beck replied that limited work had been performed using membrane filters. Other sampling technics would be investigated in the forthcoming year.

PROPOSAL

Accumulation and Storage of West Coast Shellfish Polluted with *Clostridium botulinum* Type E Spores

Storage studies on various species of West Coast shellfish purposely contaminated with spores of *Clostridium botulinum* Type E will be continued. Storage temperatures to be used in this investigation are -20 C, 10 C and 20 C. The number of spores used for pollution will be varied. The conditions necessary for outgrowth of the spores and toxin production during storage will be investigated. Contaminated shellfish will also be shucked, stored and examined. Investigation of the effects of alternate freezing and thawing of contaminated shellfish stored at -20 C is also planned. Further investigation will continue to improve methods for isolation and enumeration of *C. botulinum* Type E spores from seawater and shellfish.

COMMENTS BY PARTICIPANTS

Messrs. Dupuy and Beck discussed the merits of various storage temperatures for shellfish shellstock. Mr. Dupuy indicated that the defense mechanisms in oysters would be working at 5 C. Mr. Beck stated that in the proposed studies storage temperatures were designed to be higher than those found in commercial channels.

PROPOSAL

Studies on Wet Storage of Oyster and Clam Shellstock

Completion of the wet storage studies at the present location is anticipated early in FY67. Three additional experiments are planned during October and November.

Following these, the small laboratory float will be relocated adjacent to a commercial size sink-float at one or more plant sites in the Puget Sound area. Pacific oysters and Manila clams, if available, will be harvested at or near the plant site and placed in both floats. Samples of clams, oysters, and seawater will be taken concurrently from both floats at the low, mid, and high points of two complete tide cycles. This will be done in order to determine what effect various load capacities have on the accumulation and elimination of bacteria by shellfish. Hydrographic data such as salinity and temperature will be monitored continuously by use of a constant recording device. Experiments will be conducted on a seasonal basis.

COMMENTS BY PARTICIPANTS

Dr. Sparks and Mr. Glude discussed the ramifications of the project to date. It was suggested that the future project include parallel runs with commercial sink floats and the laboratory float. The question of bed samples was also discussed. It was agreed that where possible oyster bed samples would be included in the project.

Messrs. McMillin and Glude discussed the utilization of sink floats for commercial operations. These floats are used for cold wet storage as well as for feeding. Mr. Glude asked about the possibility of using sink floats for relaying shellfish in clean areas. Mr. Stonehouse indicated that rafting was not done in British Columbia, Canada. Mr. Neufeld discussed the possibility of contamination when sink floats were misused. Mr. Felsing suggested that one purpose of this project was to determine the effects of loading on bacterial quality. Dr. Hoff indicated that this could be determined by the continuation of the study.

PROPOSAL

Depuration of West Coast Hard-shell Clams

This project was discussed at the 1965 Research Planning Conference and at that time it was decided to use clams initially rather than oysters. Since then the project has been divided into three phases. Phase I, the determination of an optimum flow rate on a small scale model was initiated in FY66 and is scheduled to be completed within the next few months.

During Phase II, to be initiated in FY67, two scale models of a vertical and horizontal flowthrough depuration tank each with a capacity of one bushel or less will be constructed and tested. The effectiveness of each tank design in advancing the depuration process will be determined by comparing bacteriological and physical data obtained from both tank designs.

After enough background information has been obtained in Phase II, a pilot depuration plant will be constructed. Various flow rates, depuration times and operational procedures such as loading and stacking will be investigated. The effects of these and other factors on the depuration process will be evaluated.

COMMENTS BY PARTICIPANTS

Messrs Dupuy and Felsing asked about measurement of flow rates and factors affecting depuration. Mr. Vasconcelos stated that preliminary flow rate studies had been made and the measurement of turbidity and residual oxygen would be included in the study.

Dr. Quayle asked if the project could be expedited by using information available on other species of shellfish. He was particularly interested in work on the quahog on the Atlantic Coast.

Messrs Timothy and Felsing discussed the feasibility of recirculating systems. Mr. Glude and Drs Sparks and Hoff discussed the English system as described by Peter Wood. Dr. Sparks stated that Americans probably have a tendency to overdesign and may lose sight of the economics involved.

Messrs McMillin and Vasconcelos briefly discussed the alternates that could be utilized in the construction of the depuration tank.

PROPOSAL

Accumulation and Elimination of Poliovirus by the Olympia Oyster (*Ostrea lurida*)

The experiments will be carried out in a flowing seawater system using an attenuated poliovirus as the pollutant. Stocks of the virus have been prepared. Several factors were considered in selecting the Olympia oyster for these studies. This species is of commercial importance and frequently eaten raw. Previous studies using *Escherichia coli* showed that the Olympia oyster concentrated these bacteria to higher levels than did the Pacific oyster (*Crassostrea gigas*). Since the Olympia oyster is small, experiments can be carried out on a smaller scale than with larger animals, thereby reducing the quantity of stock virus needed for pollution.

In addition to the virus, *E. coli* will be used as a control pollutant to indicate whether or not the animals have been actively feeding during the experiment. Virus assay procedures will be based on studies conducted at the Northeast and Gulf Coast Shellfish Sanitation Research Centers.

COMMENTS BY PARTICIPANTS

Mr. Foster inquired about the methods to be used in measuring viruses in shellfish. Dr. Hoff indicated that methodology had been developed at the Gulf Coast and Northeast Shellfish Sanitation Research Centers. He stated that the three laboratories are now collaborating on development of one common procedure.

PROPOSAL

Cooperative Studies at the NWSSRC Site

Through the cooperative effort of the College of Fisheries, University of Washington and the U. S. Department of Commerce, further investigations in microbiology, chemistry and oceanography will be made.

A series of six recording buoys will be installed near the NWSSRC site within the next few months. The recordings will include temperature, salinity and current readings on a continuous basis. The equipment will be maintained by the Staff of NWSSL. The data will be tabulated and analyzed by the Department of Commerce. In addition, routine grab samples from the pier and at designated locations in Rich Passage will be taken. Grab samples will be tested for temperature, salinity and bacteriological determinations.

Periodic cruises will be made in the area through the cooperation of the College of Fisheries, University of Washington. Soundings, temperature, salinity and current determinations will be made at various coordinates from the pier at the site of NWSSRC. Bottom core samples will be collected to determine the proper location of salt water discharge into the estuarine area.

COMMENTS BY PARTICIPANTS

Dr. Sparks inquired about growth and mortality of shellfish located at the Manchester site. Mr. Beck stated that during 20 weeks storage oysters had shown growth and no mortality was observed. Mr. Reed asked about natural pollution in the area. Mr. Beck indicated that studies on this would be continued and that information of this type would be valuable to the new Center. These would include samples of oysters held in trays as well as shellfish samples found in the general areas.

Dr. Quayle indicated that an oceanographer should be on the staff to plan and correlate the several projects involved. Mr. Beck agreed that this was needed, but no assistance was foreseen in the immediate future. Therefore, the laboratory would have to continue to rely on other agencies for this technical assistance.

PROPOSAL

Evaluation of a Plating Technic for Enumeration of Fecal Coliform Organisms

A plating technic has been developed by the PHS Northeast Shellfish Sanitation Research Center for the direct enumeration of fecal coliform organisms. In-house evaluation of the technic will be made by three research centers within the following year. Comparative tests will be made with a 12-tube MPN method for fecal coliform organisms.

Samples of seawater and Pacific oysters, Crassostrea gigas, will be collected on a quarterly basis from three areas in the Puget Sound area. These areas will include Burley Lagoon, Glen Cove, Sinclair Inlet and Middle Point (Manchester). Oyster samples will be transferred to Sinclair Inlet and Middle Point approximately one week before sampling. These samples will be held in locked trays.

In addition to the above, approximately four market samples of Pacific oysters will be tested on a quarterly basis.

COMMENTS BY PARTICIPANTS

Mr. Neufeld stated that the use of such tables would limit the interpretation of results to only those persons having such tables available.

PROPOSAL

Research on Gonyaulax Toxins

Plans are proceeding to establish a field station in Juneau, Alaska to investigate the ecology of Gonyaulax toxins in Southeast Alaska. Studies will be initiated to determine the season distribution of the causative organisms; the relationship of these organisms to toxin production in various species of shellfish, and potential methodology in removal or neutralization of the toxin from shellfish. Within this fiscal year procurement of personnel, supplies and facilities will limit the amount of ongoing research on this project.

Lists of references on the subject have been received from most of the laboratories where research on this problem is being done and an annotated bibliography is being prepared.

COMMENTS BY PARTICIPANTS

Mr. Reed inquired if this project would have any effect on the projects at Purdy. Mr. Beck indicated that at present, persons other than those stationed at NWSSL would be assigned to the field station. Resolution of this administrative problem would be made as the project progressed.

PART III

APPENDIX

AGENDA

ANNUAL SHELLFISH SANITATION PLANNING CONFERENCE

September 19-20, 1966

Tyee Motor Inn
Olympia, Washington

Monday Afternoon - Richard VanCleve, Moderator

1:00 - 1:20 Introductions

1:20 - 1:45 Summarization of Activities - W. J. Beck

1:45 - 2:15 Summarization of Research Activities - J. C. Hoff

2:15 - 3:00 Comparative Study of Several Indicator Organisms in Samples Collected from Three Northwest Watersheds - G. J. Vasconcelos, R. D. Lee and R. F. Coene

3:00 - 3:15 Coffee Break

3:15 - 4:00 Studies on the Incidence of Clostridium botulinum Type E in Some West Coast Estuarine Areas - T. H. Ericksen

4:00 - 4:45 Accumulation and Retention of Clostridium botulinum Type E Spores in Manila clams (Tapes philippinarum) - T. H. Ericksen

6:00 - 7:00 No Host Cocktail Hour

7:00 - 8:00 Dinner

Tuesday Morning - John Glude, Moderator

9:00 - 9:45 Studies on Wet Storage of Oyster and Clam Shellstock - G. J. Vasconcelos

9:45 - 10:30 Accumulation of Bacteria by Two Species of West Coast Shellfish Maintained in an Estuarine Environment - J. C. Hoff

10:30 - 10:45 Coffee Break

10:45 - 11:15 Chemical and Biological Analysis of Shellfish and Seawater at Site of Northwest Shellfish Sanitation Research Center - W. J. Beck

11:15 - 12:00 Activities of the PHS Shellfish Sanitation Branch
- E. T. Jensen

12:00 - 1:00 No Host Lunch

Tuesday Afternoon - D. B. Quayle, Moderator

1:00 - 5:00 Research Proposals

Clostridium botulinum

Wet Storage Studies

Depuration

Pilot Plant Studies
Virus Accumulation and Elimination in Olympia Oysters

Cooperative Studies

Manchester Site
Evaluation of Fecal Coliform Plating Technic
Gonyaulax Toxins

Tuesday Evening

7:30 West Coast Shellfish Authorities Conference

Panel Discussion - "Water Quality Control Act of 1965 - Its Relationship to the National Shellfish Sanitation Control Program."

ATTENDANCE ROSTER

Eighth Northwest Shellfish Sanitation Planning Conference

Tyee Motor Inn
Olympia, Washington
September 19-20, 1966

Universities

D. Bevan	College of Fisheries, U of W.	Seattle, Washington
K. Chew	College of Fisheries, U of W.	Seattle, Washington
C. Drake	Washington State University	Pullman, Washington
J. Dupuy	College of Fisheries, U of W.	Seattle, Washington
W. McNeil	Oregon State University	Newport, Oregon
R. Northcraft	California Western University	San Diego, California
A. Sparks	College of Fisheries, U of W.	Seattle, Washington
R. VanCleve	College of Fisheries, U of W.	Seattle, Washington

State and Local Agencies

T. Blair	Oregon State Board of Health	Portland, Oregon
J. Campbell	Health Branch, Div. Lab.	Vancouver, B. C.
N. Christopherson	Wash. State Dept. of Health	Seattle, Washington
H. Foster	Calif. State Dept. of Health	Berkeley, California
D. Gatterman	Wash. State Dept. of Health	Olympia, Washington
J. Girard	Wash. State Dept. of Health	Olympia, Washington
M. Hayes	Wash. State Dept. of Health	Olympia, Washington
E. MacDonald	Wash. State Dept. of Health	Seattle, Washington
K. Michener	Oregon State Board of Health	Portland, Oregon
J. Rensel	Wash. State Pollution Control	Olympia, Washington
R. Russell	Wash. State Dept. of Health	Olympia, Washington
C. Sayce	Wash. State Dept. of Fisheries	Nahcotta, Washington
D. Snow	Marine Science Center	Newport, Oregon
C. Stonehouse	B. C. Provincial Health Dept.	Victoria, B. C.

Industry

R. Bower	Ellison Bros. Oyster Company	Olympia, Washington
E. Brenner	J. J. Brenner Oyster Company	Shelton, Washington
E. Gruble	Hilton Seafoods Company	Seattle, Washington
R. Hicks	Simmons Oyster Company	Olympia, Washington
D. McMillin	Olympia Oyster Company	Shelton, Washington
R. Nakatani	Battelle Northwest	Richland, Washington
P. Parker	Pacific Coast Oyster Growers Assn.	Tumwater, Washington
G. Pauley	Battelle Northwest	Richland, Washington
E. Timothy	Timothy Oyster Company	Ladysmith, B. C.

Federal Agencies

W. Beck	N.W. Shellfish Sanitation Lab.	Gig Harbor, Washington
R. Becker	N.W. Shellfish Sanitation Lab.	Gig Harbor, Washington
N. Bourne	Fisheries Research Bd. of Canada	Nanimo, B. C.
J. Burnett	U.S. Food and Drug Administration	Seattle, Washington
W. Clothier	Pacific Northwest Water Lab.	Corvallis, Oregon
S. Copp	Canadian National Health Dept.	Vancouver, B. C.
M. Eklund	Bureau of Commercial Fisheries	Seattle, Washington
T. Ericksen	N.W. Shellfish Sanitation Lab.	Gig Harbor, Washington
W. Felsing	USDHEW PHS Region IX	San Francisco, Calif.
J. B. Glude	Bureau of Commercial Fisheries	Seattle, Washington
J. Hoff	N.W. Shellfish Sanitation Lab.	Gig Harbor, Washington
E. Jensen	DEEFP PHS Shellfish San. Branch	Washington, D. C.
E. Kari	Federal Water Pollution Control	Portland, Oregon
L. Myers	N.W. Shellfish Sanitation Lab.	Gig Harbor, Washington
N. Neufeld	Canada Fisheries Inspection Branch	Vancouver, B. C.
D. Quayle	Fisheries Research Bd. of Canada	Nanaimo, B. C.
W. G. Styles	Canada Fisheries Inspection Branch	Vancouver, B. C.
T. Tevendale	Public Health Engineering	Vancouver, B. C.
G. Vasconcelos	N.W. Shellfish Sanitation Lab.	Gig Harbor, Washington

U.S. GOVERNMENT PRINTING OFFICE: 1967 O—255-114